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ADENOMYOMA (ENDOMETRIOMA) OF THE UMBILICUS*

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The endometrial nature of adenomyomas of the umbilicus was first suspected by Goddard[17] who reported two umbilical tumors of probable uterine origin in 1909. Cullen[7] confirmed Goddard's observations reporting umbilical tumors containing uterine mucosa, or remnants of Müller's ducts and later devoted[8] an entire chapter in his book to this subject in which he discussed fifteen cases collected from the literature; these included the three cases mentioned above. Of these fifteen cases, Cullen was in doubt about four, namely, Mintz's[32] second case and a case each by von Noordon,[44] Giannettasio,[16] and Wullstein;[47] but in the light of more recent investigations, these four cases are definitely included among the cases of endometrial growths of the umbilicus.

I have been able to collect fifty-four cases of adenomyoma, or as some prefer, endometrioma of the umbilicus. These are listed in chronological order in the table.

To this number, I add the following case:

Mrs. M. N. R., forty years old, a housewife, married twenty-three years, had one pregnancy twenty-two years ago; menstruation commenced at age fourteen and has always been normal. The balance of her history revealed nothing abnormal. About one year prior to being seen by her physician, this patient noticed drainage from the umbilicus commencing two days before the menstrual period and continuing throughout the menses. Enlargement of the umbilicus was noticed which gradually increased in size. The umbilicus was red and inflamed and bloody discharge was exuding when the patient was first seen. At the time of operation, several days later, the discharge had ceased, the redness and inflammation had subsided but the umbilicus was still enlarged. There

* Read before the Tenth Annual Convention of the American Society of Clinical Pathologists, Philadelphia, Pennsylvania, June 7–9, 1931.

ADENOMYOMA (ENDOMETRIOMA) OF THE UMBILICUS: CHRONOLOGICAL, CLINICAL AND PATHOLOGICAL DATA

CASE	AUTHOR	COUNTRY	DATE	AGE	MARITAL STATE	PREGNANCIES	MENSTRUATION	HERNIA	PELVIC PATHOLOGY	PERITONEAL ATTACHMENTS	SIZE	SWEAT GLANDS	SMOOTH MUSCLE	BLEEDING	PAIN	SWELLING	DURATION SYMPTOMS	DURATION TUMOR	REMARKS
1	Villar	F	1886	46						Pedicle attached	Egg			P				1 yr.	•
2	Wullstein	G	1893	34	M	0			Uterus	Cord not attached	3 x 1.5 cm.	P							No hair or sebaceous glands. Tumor diffuse in rt. broad ligament. Angioma and tumor of sweat glands
3	Mintz-1	G	1899	42	M			Developed after labor			Hazlenut		P	P				Few mos.	Recurrence 4½ yrs. after removal
4	Mintz-2	G	1899	38					Myomatous uterus	Attached to omentum					P	P		3 mos.	Developed 11 months later in upper end of scar
5	Mintz-3	G	1899	45											P	P		9 mos.	Skin adherent•
6	Green[19]	E	1899	50						Normal			P	P				2½ yrs.	•
7	Giannettasio	I	1900	44	M	Mt				To peritoneum	Walnut								•
8	von Noordon	G	1901	38	M	Mt						P	A					2 mos.	•
9	Ehrlich	G	1909	54	M	0			Uterus removed 10 yrs. before		3 cm.	P	A						Tumor of sweat glands. Bilateral mammary carcinoma
10	Goddard-1	U. S.	1909	44	S	0	Normal			To rectus	2 cm.	P	P		P	P	1 yr.	2 mos.	
11	Goddard-2	U. S.	1909	42	M	4	Normal	None	Normal	Abdomen opened, normal	2 cm.	P	P	P	A	A	6 yrs.		Obese
12	Herzenberg	G	1909	36					Fibro-adenoma of the uterus 1 mo. before		2.5 cm.			P	P	P	1 mo.	1 mo.	Upper end of laparotomy scar
13	Cullen	U. S.	1912	38	M	4	Regular				1.5 cm.	P	P		P		2 yrs.		•
14	Waegeler	G	1913	48				None		To peritoneum		P	P	A	P	P		1 yr.	Obese
15	Zitronblatt[48]	G	1913	36						Normal	Hazlenut			P				2 yrs.	•
16	Barker	E	1913	37				None		Abdomen opened, normal	Filbert		P					Few mos.	Recurrence 4½ yrs. at left anterior superior spine of ilium•

17	Jones	U. S.	1913								0.75 in.	P							•
18	Lindau[29]-1	G	1914								17 x 20 mm.	P		P	P	P	1½ yrs.		Above and to left of umbilicus
19	Lindau-2	G	1914	34	S	0					18 x 20 mm.		A		P	P			Left and above
20	Mathias	G	1920	40									P						Goblet and bony spindle cells*
21	Guthrie[9]	U. S.	1920	46	M	2	Normal				1.5 cm.		P	A	P	P		2 yrs.	•
22	Adams[9]	U. S.	1920	37							1.3 cm.	P	P	A	P	P		1 yr.	•
23	Mahle and MacCarty	U. S.	1920	42	M	4		Umbilical hernia		Abdomen opened, normal				P	P			4 yrs.	Growth not attached to umbilical hernia*
24	Keitler	G	1923	45	M	2	Normal	Nut-size, livid	Normal	Abdomen opened, normal				P	P				•
25	Lauche-1	G	1923	48	M	1	Normal						A						•
26	Lauche-2	G	1923	38						Abdomen opened, normal	Walnut	P						1½ yrs.	•
27	Lauche-3	G	1923	50	M	5		Small umbilical at last pregancy			3 marks		A			P		1½ yrs.	This case also reported by Schneider and by Ribbert*
28	Tobler-Case 6	G	1923	27							2.5 cm.	P	A						Giant cells. (Langhans?)
29	Tobler-Case 7	G	1923	44					Fibro-myomatous uterus, cysts of ovaries and tubes, growth on cervix	Tumor through wall attached to peritoneum by fossette	2 cm.	P	P	A	P	P	3 yrs.		Serial sections show glands coming from peritoneum
30	Andrews	E	1925	44	M	0	Painful and profuse 3 yrs.	None	Tender mass, Douglas pouch		Walnut		P	P	P	P	3 yrs.	1 yr.	Urachus normal
31	Schiffman and Seyfert	G	1925	57	M	1	Irregular 4 yrs.		Papillary cystoma rt. ovary, plaque Douglas pouch	Normal	Walnut	P		P	P	P		10 yrs.	Emaciated. Hair follicles and few islands decidual and giant cells
32	Edwards and Spencer	U. S.	1925	45	M	3	Normal		Normal		2.5 cm.	P	P	P	P	P	9 yrs.	7 yrs.	Obese

Key: G—Germany, U. S.—United States, F—France, I—Italy, Den.—Denmark, Scan.—Scandinavia, B. A.—Buenos Aires, H—Holland, E—England, P—present, M—married, S—single, Mt. multipara, ⁂—four miscarriages, †—abortion, A—absent, •—no clinical history.

ADENOMYOMA (ENDOMETRIOMA) OF THE UMBILICUS: CHRONOLOGICAL, CLINICAL AND PATHOLOGICAL DATA—*Concluded*

CASE	AUTHOR	COUNTRY	DATE	AGE	MARITAL STATE	PREGNANCIES	MENSTRUATION	HERNIA	PELVIC PATHOLOGY	PERITONEAL ATTACHMENTS	SIZE	SWEAT GLANDS	SMOOTH MUSCLE	BLEEDING	PAIN	SWELLING	DURATION SYMPTOMS	DURATION TUMOR	REMARKS
33	Stacy et al.	U. S.	1926	39	M	0	Increase 3 yrs.	None	Multiple fibromyomata uterus					P	P		1 yr.		Negro. Degeneration of some of the fibromyomata
34	Anglessio	I	1926	34	M	1			Cyst of ovary 2 yrs. prior				P	P	P	P		1 yr.	•
35	Weller[46]-1	U. S.	1927	49									A	P					•
36	Weller-2	U. S.	1927	45									A	A					•
37	Oberling and Hickel[35]	F	1927	44							Cherry				P	P	Since childhood	6 mos.	
38	Steiner[11]	G	1927	46	M	Mt	Normal								P	P		1 yr.	•
39	Palmen-1	Scan.	1927	35	S	0	Normal		Normal	Abdomen opened, to rectus and peritoneum	Hazlenut	A	P		P	P		6 mos.	Meckel's diverticulum present, not attached to tumor
40	Palmen-2	Scan.	1927	48	M	5	Normal			To peritoneum	Egg			A	P	P		15 yrs.	Small opening into tumor from peritoneal surface. Obese
41	Palmen-3	Scan.	1927	33	M	2	Normal			To peritoneum and skin	Walnut			P	P	P		2 yrs.	
42	Lelievre and Montpellier[28]	F	1927																•
43	Kohler	G	1927	46	M	0	Normal			Pedicle at base not attached to peritoneum. Abdomen opened, normal				P	P	P		5 mos.	•
44	Foderl	G	1927	38	M	1	Normal			Cord not attached to peritoneum	Hazlenut	P		P	P	P		3 yrs.	•

45	Baltzer	G	1927	41	M	2			Myomatous uterus	Abdomen opened, numerous growths through abdominal wall; not attached to peritoneum	Hen egg			P	P	P		3 yrs.	Obese. Similar growths on left ovary, appendix, peritoneum and sigmoid
46	Busser	H	1928	30						Abdomen opened, normal				P				2 yrs.	*
47	Roques	E	1928	49	M	8 *		Right inguinal hernia at 15		Not connected to peritoneum. Abdomen opened, normal. Sinus in peritoneal surface	17 x 15 mm.		P	P	P	P		4 mos.	
48	Baltzer	G	1929	40	M	1	Recently irregular			Abdomen opened, numerous growths	Pear	A		P				8 weeks	On peritoneal surface, sigmoid, and in pelvis
49	Frero	B. A.	1929	55	M	5	Normal		Large uterus. Ulceration on anterior lip of cervix	Fossette at internal surface				P	P	P		1 yr. or more	Both tubes contained about 10 cc. dark, old blood. Died 6 days after operation
50	Holm-1	Den.	1930	43	S	0			Normal	To peritoneum and sinus into tumor	Hazlenut					P		Some time	
51	Holm-2	Den.	1930	29		1†				Recurrence, adherent to omentum and peritoneum	Small nodule		P						Recurred in two months. Metastases, left supraclavicular region, right inguinal region. Died within a year. Post mortem shows numerous growths through pelvis, liver, etc.
52	Enzer	U. S.	1930	18			Normal			To peritoneum. Abdomen not opened	2.5 cm.		P	P	A			6 mos.	Serial sections show glands coming from peritoneum. Two glands composed of goblet cells
53	Keene and Kimbrough	U. S.	1930																Similar growths in both ovaries, none in anterior abdominal wall
54	Keene and Kimbrough	U. S.	1930							Abdomen opened, normal									
55	Spitz	U. S.	1931	40	M	1	Normal		Clear	Normal	5 x 3 cm.	P	P	P	P	P		1 yr.	

FIG. 1. EXTERNAL AND UPPER SURFACE OF TUMOR

FIG. 2. CUT SURFACE OF TUMOR SHOWING SMALL CYSTS FILLED WITH OLD MENSTRUAL BLOOD

was no pigmentation nor could any pelvic or abdominal pathology be found. The blood and urine were normal.

The specimen (fig. 1) sent to me by Dr. Watt Yeiser of Columbia, Tennessee, was approximately 5 cm. in its greatest length, that is from its epidermal surface to its base and approximately 3 cm. in its greatest diameter. The upper surface (fig. 1) showed an elevated mass about 1.5 cm. in diameter which projected above the level of the surrounding collar of skin for 15 mm. This surface

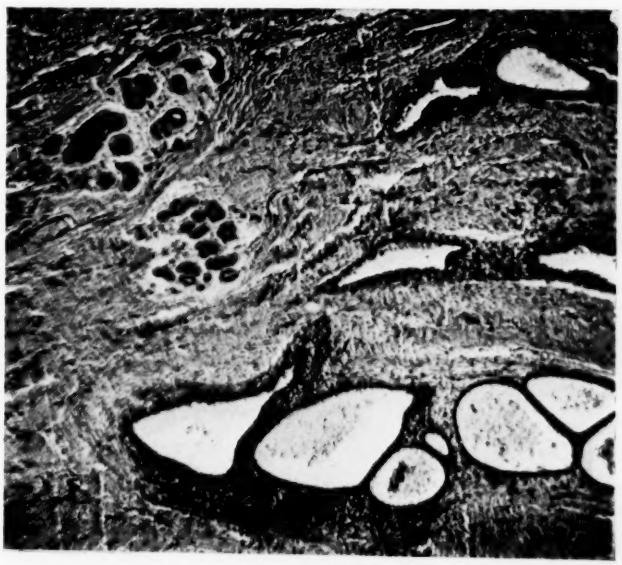

FIG. 3. PHOTOMICROGRAPH OF SECTION OF TUMOR

In the upper left hand corner note normal sweat glands. Endometrium-like glands surrounded by cytogenous tissue are seen in the upper right hand portion. The lower right hand portion shows a chain of endometrial glands undergoing cystic dilatation. Note absence of cytogenous tissue around the glands in the lower right hand corner and note contents of glands.

was covered by a dirty grayish exudate in which there were scattered a few irregularly shaped, small sized brownish masses. The sides and base of the specimen simply showed the cut surface of the surrounding connective tissue. The mass was bisected and the cut surface (fig. 2) showed from above downwards the epidermis which appeared normal. About 2 mm. beneath this, there was a rather even row of dark reddish to dark brown colored areas, varying in size from less than 1 mm. up to 3 mm. in diameter. Sections through other portions of the tissue, showed some of the above brownish areas to be prolonged down-

wards in a tubular cystic formation and from which a small amount of brownish exudate escaped. Coarse bands of connective tissue traversed the specimen in a fan-like arrangement and scattered throughout the specimen were additional brownish areas. The matrix of the specimen was smooth, creamy white and glistening.

The histological structure showed a normal epidermis which has thickened in some areas. Beneath this (figs. 3, 4 and 5), merging imperceptibly into the tumor mass were interlacing bundles and strands of connective tissue, in the meshes of which were located numerous glandlike structures which varied widely in size and shape. They were cut in cross, oblique, and longitudinal sections. Some were very small, others assumed cyst-like dimensions (fig. 4); they occurred singly, in groups of three and four or more and in chains. Many of these gland-like areas were surrounded by a heavily stained zone of round cells which contained large round nuclei. Towards the periphery of this zone of round cells, spindle-shaped cells were seen to emerge. These were arranged in small strands and groups, in cross, oblique, and longitudinal sections and blended into the surrounding connective tissue stroma. These spindle-shaped cells resemble non-striated muscle.

Immediately beneath the epidermis were several groups of small, round glands, uniform in size and shape. These were lined with cuboidal epithelium. These glands were enmeshed in a connective tissue stroma and showed no evidence of hyperplasia or hypertrophy. They were definitely normal sweat glands (fig. 3). No hair follicles were seen; an occasional mass of round cell infiltration (lymphoid cells?) was seen throughout the entire specimen.

Further consideration of the histo-pathology will be described in greater detail under the general discussion.

GENERAL DISCUSSION

Adenomyomas of the umbilicus are easily recognized new growths. They occur exclusively in women* and as a rule have

* Koslowski[26] reported a case of true adenoma of the umbilicus in a man 55 years of age. Due to the fact that several previous investigators have included this case in their group of umbilical adenomyomas, it is advisable to point out that this tumor does not conform to any of the descriptions given of these cases. The growth was located half way between the umbilicus and the symphysis. It, of course, had none of the physiological symptoms of pain, swelling and bleeding during the menstrual period (?), and the author called his growth fibro-adenoma-sub-malignun. Cullen[8] considered this growth in connection with the growths arising from the omphello-mesenteric duct, but doubts this possibility. He again considered this same case in connection with tumors of the urachus, where he finally placed it. Lauche also takes exception to including Koslowski's case with the adenomyomas of the umbilicus.

the following characteristic features: they occur during the menstrual life; are small, slowly growing, becoming swollen, tender, cyanotic and in about half of the cases discharge bloody fluid during the menstrual period. These symptoms subside with the cessation of menstruation only to reappear at the next cycle.

Histologically, these growths are characterized by containing numerous glands embedded in a cellular (cytogenous) stroma, having the identical appearance of the uterine mucosa. Old

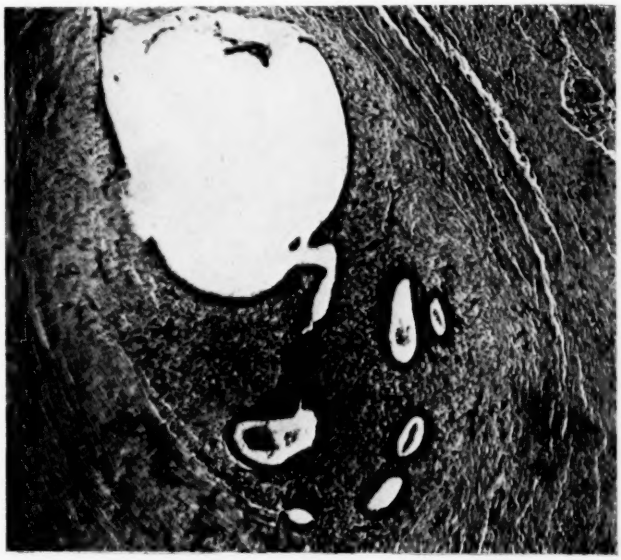

FIG. 4. PHOTOMICROGRAPH OF SECTION OF TUMOR

Note cytogenous tissue around small glands and absence of this tissue around the cystic gland. Also note communicating duct between cyst and smaller gland.

blood pigments, desquamated cells and debris are found in the gland spaces, especially those that are becoming cystic; extravasated blood is also seen in extra-glandular tissue spaces.

The exact origin of these growths is not known and a voluminous literature has accumulated as a result of the arguments supporting the various theories proposed to establish their origin.

These growths are similar in nature to endometrial growths found in other localities; namely, in the wall of the uterus, the ovaries, recto-vaginal septum, tubes, round ligaments, sigmoid flexure, wall of appendix and small intestine, rectus muscle, abdominal scars following Caeserian sections, certain laparotomies, and in the inguinal region and the umbilicus. Collectively, these growths have been called endometriosis. Mintz

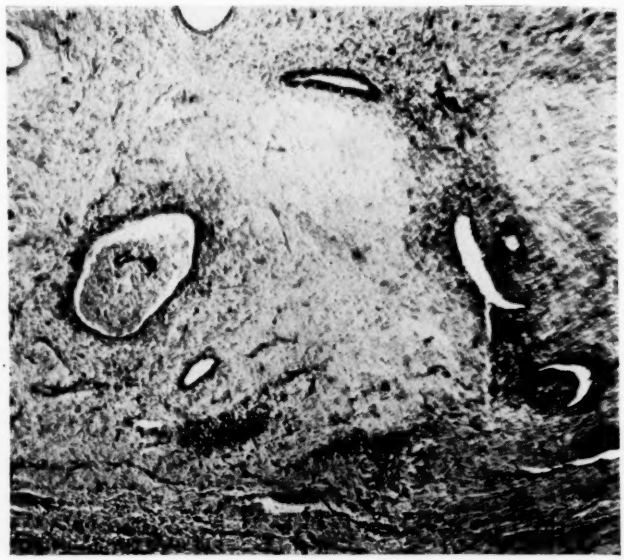

FIG. 5. PHOTOMICROGRAPH OF SECTION OF TUMOR

Note bifurcation of gland resembling immature uterus (first described by Cullen). Lymphocytes are in the lower portion of the field and the contents of the cystic gland contains a strip of desquamated lining.

first described the growths in the umbilicus as true adenoma of the umbilicus. When involuntary muscle was identified in these growths, the term adenomyoma was applied. Blair Bell first applied the term endometrioma and this seems to be the most appropriate name. Other designations, such as fibro-adenomyosis, muellerianensis, adenomyositis, seroepitheliale adenoma, choristoblastoma seroepitheliale, and others have been used according to the personal preference of the writer. I prefer the

term endometrioma because it describes the true character of the tissue.

GEOGRAPHICAL DISTRIBUTION OF UMBILICAL ENDOMETRIOMA

Fifteen cases including my own have been reported in the United States, four cases have been reported from England, twenty-four from Germany, three each from France and Sweden, two each from Italy and Denmark and one each from Holland and Argentina.

I have not found any cases reported from the Orient, the Far East, or Africa, although numerous cases of endometriosis of the abdominal cavity and pelvic organs are reported from all quarters of the globe.

The case reported by Stacy[40] et al. was in a negro woman; all others were apparently white women.

AGE INCIDENCE

Enzer's[12] case, a girl eighteen years old is the youngest on record. The other cases reported were of the following ages: two, between twenty and thirty years of age; sixteen between the ages of thirty and forty; twenty six between the ages of forty and fifty; two patients were fifty, one each was fifty-four, fifty-five and fifty-seven. The age of the patient was not recorded in five instances.

MARITAL STATE

The clinical histories are incomplete in regard to the marital state of the majority of patients. Of the twenty-seven married women, five bore no children, seven bore one child each, four bore two children each, one gave birth to three children, three had four children each, three had five children each and one gave birth to eight children. It was stated in the case of three other patients that they were multiparas.

MENSTRUATION

Sixteen authors stated that menstruation had always been normal. Irregularity attended with pain and profuse bleeding

for three years was reported by Andrews;[1] Schiffman and Seyfert[38] reported four years with pain and profuse bleeding; Stacy et al., 3 years; Baltzer's[4] second case, recent irregularity. Holm's[21] second case, had one abortion; Roques,[37] had four miscarriages. The other case records contain no data in regard to the menstrual condition.

The majority of writers make no observation about their patient's constitutional resistance. Goddard's second case, Edwards and Spencer's,[10] Baltzer's[3] first case, Palmen's[36] second case, and Waegeler's[45] case, were obese. Schiffman and Seyfert's case was emaciated.

HERNIAS

Mintz's first case developed an umbilical hernia shortly after pregnancy and the umbilical growth commenced ten years later. Keitler[24] reported a nut-sized livid hernia. Roques reported a right inguinal hernia, at the age of 15, thirty-four years previous to the development of the tumor. Lauche's[27] third case, which is also reported by Ribbert and Schneider in 1916, had a small umbilical hernia which developed at the last pregnancy. Mahle and MacCarty[30] report an umbilical hernia and state that the tumor was not attached to it. The absence of a hernia was specifically noted by Goddard in his second case, by Barker,[5] Stacy et al., Schiffman and Seyfert and Waegeler. The others do not mention hernia.

ABDOMINAL AND PELVIC GROWTHS

Baltzer found generalized endometriosis in both of his cases. The first case had growths on the peritoneum, appendix, sigmoid and left ovary; a myomatous uterus was also present. His second case had similar growths on the peritoneum, sigmoid and in the pelvis. As a result of these observations, he strongly advised that the abdominal cavity be thoroughly explored in every case.

The abdomen was opened in Mintz's second case in which a myomatous uterus was found and an umbilical growth developed eleven months later; it was firmly attached to the omentum.

Ehrlich's[11] case had a uterus removed ten years before the growth developed. Wullstein's case had a pelvic tumor the size of a fist connected to the uterus and diffuse growth through the right broad ligament. The umbilical growth had a pedicle extending into the abdominal cavity. This was not connected to the pelvic growth. Herzenberg's[20] case had a fibro-adenoma of the uterus. The umbilical growth developed one month later in the upper end of the laparotomy scar. Andrew's case had a fixed tender mass in Douglas' pouch. The umbilical growth was not connected to this mass but sections from both growths showed the same structure. Schiffman and Seyfert's case had a cysto-papilloma of the right ovary and a white, flat, elevated plaque in Douglas' pouch. There was no connection to the umbilical growth. Anglesio's[2] case had a cystic ovary removed two years before the appearance of the umbilical growth. The case reported by Stacy et al. had large multiple fibromyomas of the uterus, some of which were degenerating and adherent in the pelvis. The umbilical growth was not attached. Frero's[15] case showed an ulceration of the anterior lip of the cervix; the uterus was large and hard and both tubes contained old, dark blood; they were adherent to the posterior face of the isthmus and Douglas' pouch; the umbilical growth was not attached. Holm's second case, recurred within two months and developed masses in the right inguinal region and the left supraclavicular fossa. Sections were the same as in the original growth; the patient died within a year and similar growths were found in the liver, posterior mesenteric glands, along the left ureter (which was greatly dilated above the point of pressure by the growth), a mass the size of a fist in the supraclavicular fossa, in the groin, and the umbilical growth had grown through the entire thickness of the abdominal wall and was adherent to the omentum and peritoneum. Tobler's[42] seventh case had numerous small cysts of both tubes and ovaries and multiple fibromyomas of the uterus. Keene and Kimbrough's[23] first case had similar growths on both ovaries.

Fraser[14] reported an umbilical endometrioma with twenty-three distinct similar growths in various places in the pelvis and peritoneal cavity in a monkey, *Macacus rhesus*. This is very

similar to the two cases in humans reported by Baltzer. It may also be noted here, that generalized endometriosis in the peritoneal cavity without an umbilical growth has been reported by several writers.

The abdomen was opened in cases reported by Goddard (second case), Barker,[5] Mahle and MacCarty, Keitler, Kohler,[25] Roques, Busser et al.,[6] Lauche (second case), and Keene and Kimbrough with normal findings. Palmen's first case had a small Meckel's diverticulum which was not attached to the umbilical tumor.

PERITONEAL ATTACHMENT

Attachments to the peritoneum by means of cords or stalks were reported by Villar,[43] Foderl,[13] Palmen in all three of his cases, Waegeler, and Holm's first case. It was connected to the omentum in Mintz's second case, and in Holm's second case. Stalks or cords were present which penetrated the abdominal wall to the peritoneum but were not attached in Wullstein's, Foderl's and Kohler's cases. In Palmen's first case, in which the small Meckel's diverticulum was present, the umbilical growth was attached to the peritoneum and rectus sheath; in his third case the growth was adherent to the skin and peritoneum. The growth occupied the entire thickness of the abdominal wall in Baltzer's first case, Holm's second case, and Tobler's seventh case.

DURATION OF SYMPTOMS AND TUMORS

Symptoms such as uneasy feeling, itching, pain, tenderness, swelling, redness and cyanosis were present in a number of cases prior to the development of the tumor. The shortest length of time reported was two months while the longest was nine years. Palmen, in his second case, reported symptoms present since childhood, the patient being forty-eight years old at the time of operation.

The tumor was noticed as early as one month prior to operation in one case. In thirteen cases prior to one year; in fourteen cases, one to two years prior; in four cases, two to five years prior; in

one case, seven years prior; in one, ten years and in one, fifteen years prior.

There was a recurrence after four and a half years in Mintz's first case in the umbilical scar. In Barker's case, four and a half years after removal of the umbilical growth, a nodule appeared at the anterior superior spine of the ileum. In Holm's second case, within two months the growth recurred locally and metastases were present in the right inguinal region and left supraclavicular fossa.

Pain, swelling and discharge of bloody fluid were present in the majority of cases. Twenty-five had discharge of bloody fluid from the growth. Six had discharge of sanguinous fluid without attendant pain and swelling. In sixteen cases pain and swelling was present without discharge. The appearance of these symptoms was not regular. In some the discharge would appear at regular monthly intervals while in others it might be profuse one month and absent for several months and then reappear. Some would have pain and swelling without bleeding and the following month have the discharge without pain and swelling. The majority would notice pain and swelling with the development of redness or cyanosis around the umbilical scar for several days prior to the onset of menstruation followed by discharge of varying amounts from the growth, from a few drops to several teaspoonsful, of light stained to almost black blood. The discharge would subside with the menstrual flow (some discharging a varying number of times), this would be followed by the disappearance of the swelling, redness or other discoloration, and pain. In some, a greenish discoloration of the umbilical region would remain for several days. After a period of quiescence, the cycle would commence again.

GROSS APPEARANCE OF THE GROWTH

The majority reported a single nodule or tumor lying deep in the umbilical scar, filling it and in some cases projecting to a variable distance (1 or 2 cm.) above the level of the surrounding tissues. Several reported two nodules and a papillomatous appearance. Pinpoint openings, through which the discharge is

seen to escape, are reported by some. Several reported a thin, tissue paper like covering over a cyst like cavity, through which the blood contents of the cavity can be seen. The shape of the tumor is not definite. None are encapsulated; the majority merge gradually with the surrounding connective tissue, although in some the tumorous mass can be more readily identified. Some are covered with a dry, bloody secretion; my case was covered with a purulent like exudate. As noted above on peritoneal attachments, some of these growths occupy the entire thickness of the abdominal wall. As a rule, they are movable; it was firmly adherent to the skin in only two instances (Palmen's third case, and Mintz's third case). Even when attached to the rectus, they are still movable.

Some of these growths were excentric and appeared to one side or the other of the umbilical scar. In several cases they were located in the upper end of the laparotomy or hernial scar. Several specimens had definite sinuses at the upper or in the lower poles and as noted above, cord-like attachments to the peritoneal surface were present in a few of the cases. The cut surface of the growth was described almost uniformly as consisting of a pearly or creamy grayish appearance with coarse trabeculae or fasciculae of radiating, for example, fan shaped bands of connective tissue coarsing through the specimen. Brownish or reddish brown areas were seen scattered throughout the specimen, few to numerous, small pinpoint to several millimeters in size. In several cases larger cyst like cavities were noted. Sinuses running for considerable distances through the specimen were noted in a few cases.

HISTOLOGICAL APPEARANCE

The histological appearance is characteristic and with few exceptions, which will be noted, are identical in all specimens. Photomicrographs accompany many of the articles and an endometrial like appearance is clearly shown. The skin is hypertrophied, the papillae deepened, the basement membrane smooth and intact. Brownish pigment and deposits of red blood are frequently reported in the deeper layers of the epidermis. Fibrous connective tissue, arranged in course bands and in fine fascicula-

tion, traverse the sections. At numerous places gland like structures appear. These vary in size from a pinpoint to cystic dimensions. Smaller glands are frequently arranged in groups or chains and cysts are frequently seen lying side by side. The glands are seen in cross, oblique and longitudinal sections. These glands are surrounded by a compact cellular stroma composed of round and spindle-shaped cells with large, round or oval, deeply staining nuclei. This stroma, in various places, blends gradually with the surrounding fibrous connective tissue in some, while in others, the line of demarkation between the two types of tissue is clear cut.

This typical stroma was first called "cytogenous" by Ribbert and practically all of the German writers have adopted this term to describe the stroma surrounding the glands. This stroma is also interesting in that it bears a definite relation to the glands and will be referred to later.

The lining of the glands varies with the size and cystic proportions. The small glands are lined with high cylindrical epithelium with the nucleus situated from the middle to the base of the cell and many of these cells have cilia. As the glands get larger, that is become cystic, the lining cells first become cuboidal and in the largest cysts, the lining cells are flat. Many of these cystic areas contain (fig. 5) desquamated epithelial cells, some of which are of recent origin, while others are old. They also contain old and fresh blood, blood crystals and amorphous debris. Other cysts are empty. Most of the writers interpret the change and the shape of the lining cells as being due to pressure from the cyst contents. Occasionally, a group or chain of glands are seen which gradually increase in size and a communicating duct can be seen between some of them (fig. 4). The "cytogenous" stroma, is especially well developed and present in large masses entirely surrounding the smaller glands, which are lined with cylindric epithelium, but as the epithelium assumes the flatter shape, the "cytogenous" stroma gradually becomes thinner and thinner, until it is entirely absent surrounding the portion of the cystic spaces lined with flat epithelium. Ribbert refers to this condition as the "floor of the cyst resting upon a foundation of

heavy cytogenous stroma, while the roof has only fibrous connective tissue to cover it." Capillaries and larger sized blood vessels, together with lymph spaces are present. In many, small to larger masses of lymphocytes are present. Evidence of old menstrual blood and also of recent hemorrhage is found in many portions of the tissue.

Two other elements are frequently present: these are sweat glands (fig. 3) and involuntary or smooth muscle from which the tumor derives a portion of its name. Cullen says, "the normal umbilical scar is covered with a very thin squamous epithelium and is devoid of hair follicles, sweat glands and sebaceous glands."

Of the twenty-five cases having a discharge smooth muscle and sweat glands were present in four, smooth muscle alone was present in eight, sweat glands alone were present in three, neither smooth muscle nor sweat glands present in ten. Sweat glands and smooth muscle without discharge were present in five. It should be remembered that microscopic evidence of either old or fresh hemorrhage, usually both, was present in almost every instance.

In Wullstein's case an angioma was present, with a marked increase of sweat glands; hair follicles and sebaceous glands were absent. Mintz's second case contained collagen fibers. Ehrlich's case had a tumor of the sweat glands; he specifically states goblet cells are not present. Mathias'[31] case contained a few goblet cells and some bony spindle cells. Schiffman and Seyfert reported hair follicles, a few islands of decidual cells and a few giant cells. Enzer[12] reported two glands consisting entirely of goblet cells. Tobler (case 6) reported granulation tissue and foreign body giant cells, which reminded him of Langhan's cells. Holm (second case) reported occasional mitotic figures in the sections from his post mortem material; nowhere could he discover a breaking through of the basement membrane; sections from all the areas (umbilical, inguinal region, supraclavicular region, omentum, liver, et cetera) showed the typical endometrial structure.

THEORIES REGARDING ORIGIN

The earlier writers considered the remains of the omphellomesenteric duct as being the source. Several speak of the rem-

nants of the vitalline duct and of Müller's duct, this later source being especially favored by Cullen.[7] Sampson[39] in studying the entire question of endometriosis, reported his findings as a result of numerous observations and advanced the Theory of Implantation. He divides the tissue into four, or possibly, five groups.

(1) Direct or primary. These result from direct invasion from the uterine wall by the uterine mucosa. These are the uterine adenomyomas.

(2) Peritoneal implantation from retrograde menstruation. These spread like cancer and then invade the underlying structures. (It seems that Baltzer's two cases might be included in this group.)

(3) Transplantation. Those that appear in abdominal scars after Caeserian section and other operations upon the uterus and tubes and after certain herniotomies. (Such growths have appeared in abdominal scars after appendectomies. Certainly, no source from the uterus or adnexa can be concerned here.)

(4) Metastatic. These are extraperitoneal and spread like cancer, possibly through the lymph and venous channels.

(5) Developmentally displaced. Though he admits the possibility, he has never seen it.

At the meeting of the American Gynecological Society, in 1925 Sampson stated:

Furthermore, this misplaced endometrial tissue is governed by the same natural laws, in its reaction to menstruation, pregnancy and the menopause, as the mucosa lining the uterine cavity. On the basis of its histological structure and physiological function, we must conclude that this tissue is as truely 'Müllerian' as that arising in the uterine wall from its direct invasion by the uterine mucosa.

Much opposition has arisen to many of Sampson's contentions. Lauche advanced the theory of peritoneal or serosal source for these various endometrial growths and he is ably supported by Nicholson,[34] and others. At the present time the question remains unsettled.

In the case of the umbilical endometriomas, I feel that Sampson's theory is untenable. I cannot conceive of these growths occurring without having some demonstrable connecting link with the parent source. Granting that laparotomies were performed in Mintz's second case, three years prior, in Ehrlich's case ten years prior, in Herzenberg's case one month prior, and that herniotomy was done ten years prior in Mintz's first case, thirty-

four years prior in Roques's case, and ovariotomy was performed two years prior in Anglesio's case, I can see no possible connection nor do these authors see any connection between these operations and the subsequent development of the umbilical growth.

As regards the transplantation theory, it of course, can be considered only in connection with operations upon the uterus and tubes. Unless the uterus is opened, as in the case of Caeserian section, I cannot conceive of the endometrial cells from the uterine mucosa reaching the umbilicus. I certainly agree with Lauche, Nicholson and others who take strong exception to this possibility. Considering the peritoneal fossettes as described by Cullen[8] the presence of which is reported by Frero, Roques, Tobler, and Enzer; the cords which were attached to the peritoneum as reported by Villar, Wullstein, Palmen's (third case), Kohler, Foderl and the presence of sinuses by Roques and Holm, one must consider the greater likelihood of these growths arising from peritoneal cells.

By means of serial sections Tobler and Enzer described the source of the endometrial like glands in their umbilical growths as coming directly from the peritoneum. Indeed, it has been shown by Nicholson, Lauche, and others, that the peritoneum is directly connected to these growths in every situation in which they have been found, possibly excepting the uterine adenomyoma, and here even, the possibility is by no means remote.

Lauche, Nicholson, Baltzer,[3] Foderl, Schiffman and Seyfert and others contend that the presence of ovarian hormones is necessary for these growths to develop. They occur only in sexually mature women and the physiological symptoms of swelling, pain and bleeding are present only during the menstrual cycle. The spontaneous disappearance of endometrial growths from the pelvis and bladder after removal of the ovaries has been reported by Graves[18] and Keene.[22]

Neumann[33] transplanted endometrium of the rabbit into the peritoneum of the same animal and of other animals from the same litter. These transplants formed cysts when ovarian hormones were present. When castration had been done a few weeks before operation, no cysts were formed and the transplants were resorbed. Transplantation into males was unsuccessful.

SUMMARY

(1) An additional case of adenomyoma of the umbilicus is reported.

(2) The geographical distribution of all the cases heretofore reported is given and the cases are arranged in chronological order.

(3) Age, menstrual history, marital state, number of pregnancies, the association of other tumors and previous operations of each case, are considered.

(4) Clinical symptoms, such as pain, swelling, discoloration, discharge and size of growth are tabulated; as are also the histological findings in regard to the endometrial like glands, smooth muscle, sweat glands and other constituents of the growth.

(5) Some of the various theories of origin are considered.

(6) Those cases showing definite cord like attachments or other direct evidence of attachment to the peritoneum are discussed in connection with the serosal theory of origin.

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THE SPECIFICITY OF BACTERIAL ALLERGY*

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The resemblance between the two phenomena appeared at first to justify the idea that allergy in human beings is identical with experimental anaphylaxis. But as studies progressed certain discrepancies between the two were observed and the doctrine that clinical allergy was basically different from anaphylaxis gained rather wide acceptance. The pendulum is however swinging back and with increasing evidence there appears more and more justification for the assumption that the two conditions are the same.

Curiously enough, somewhat the reverse of this sequence is taking place with regard to bacterial allergy. Much of the groundwork on anaphylaxis had been done with bacterial antigens[6, 8, 13, 15] and the conception of sensitization to foods, pollens and the like grew out of the analogy to bacterial anaphylaxis. Recent observations on bacterial allergy indicate however a difference in the type of reaction which cannot yet be entirely fitted in to the familiar picture of anaphylaxis as we have known it in the laboratory.

Typical anaphylactic phenomena including acute anaphylactic shock, passive sensitization, antibodies and precipitins in the blood, contraction of the sensitizied uterus and antianaphylaxis, may be produced with bacterial antigens. But two observations which have of late received especial study remain to be entirely satisfactorily fitted into the picture. These are the tuberculin type of reaction and the discovery of the soluble specific carbohydrate substance described by Heidelberger and Avery.[5]

That the tuberculin reaction is not dependent solely on the

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presence of antibodies in the sensitized animal is indicated by the fact that it is only present in animals which in addition to being sensitized also carry or have carried active tuberculous infection. It has therefore been postulated that the delayed, twenty-four hour reaction to bacterial substance or extractives such as tuberculin depends upon the existence of a socalled "third substance" of as yet unknown nature which is manufactured by the tissues at the site of the local infection.[16] The delayed positive reaction to antigenic bacterial substance therefore indicates not only an actual sensitization but also an infection, with some as yet underdetermined specific reaction between host and parasite.

The "soluble specific substance" appears to be a complex carbohydrate, different for different bacteria, and responsible for the specificity of bacterial antigen. The specificity appears to be so closely bound up with the carbohydrate moiety that the latter alone, freed from protein will produce a typical immediate skin reaction, with wheal and erythema, in sensitized animals. This substance apparently determines the specificity of the immune reaction. It combines with antibodies, yet it appears to have no antigenic power. It is unable to initiate the production of antibodies. It therefore appears to be a true haptene. It is a factor in sensitization and immunity and is apparently responsible for specificity, but, alone, it will not produce sensitization. To do this it must be combined with the protein fraction of the bacterial cell.

We must conclude that in bacterial sensitization we are studying a distinctly more complex problem than that of allergy to the usual exogenous allergens; that we are dealing with substances and reactions of whose nature we are still rather ignorant. With these facts in mind, we are in a better position to analyze the clinical observations that have been made for and against bacterial specificity.

THE CLINICAL ALLERGIES

Among the leading protagonists of specific bacterial allergy we should mention Thomas, Famulener and Touart[12] and Brown.[1] The former believe in the specificity of the skin reaction to bac-

teria and, using bacteria to which positive skin reactions have been obtained they report complete relief in 51 per cent of their bacterial allergics and 39 per cent of partial relief. Brown reports 65 per cent complete or practically complete relief in his bacterial asthmatics. Rackemann[8] reports nearly as good results, with 18 per cent cured and 52 per cent improved, but he is by no means as certain that the reaction and the improvement are based on specific bacterial sensitization.

In my own investigation of bacterial allergens I have in general followed Famulener's technic of cultivation in dextrose broth and on blood agar and Huntoon's medium, methods which tend somewhat to promote differential growth of the streptococci, pneumococci and gram-negative organisms, with subsequent plating and pure culture isolation. At the same time I make a group isolation of those organisms which will survive twenty-four hours in the patient's clotted blood, following the Solis-Cohen selective pathogen technic. The latter in our experience appears to favor the growth of the streptococci although not exclusively. The patient is then tested intracutaneously with the differential pure culture vaccines and the pathogen mixture. Our experience coincides with that of Thomas and his collaborators and of Rackemann, and of Walker,[14] that the most frequent positive reaction is the delayed reaction of inflammatory type, appearing within twenty-four hours, but that occasionally one observes a characteristic immediate reaction with wheal and erythema.

Our results have however not been as satisfactory on the whole as some of those reported, in that in those cases showing positive prompt or delayed reactions attempts at desensitization often fail to give relief. In these same cases subsequent treatment with a pooled vaccine, a hodgepodge of those vaccines which have helped in other cases, has sometimes given relief. Furthermore as good results are sometimes obtained by such nonspecific methods as the subcutaneous injection of peptone solution.

SPECIFICITY

Such observations naturally raise the question as to the specificity of the reaction even when improvement results. Of course one must realize that in these studies we are dealing with a par-

ticularly difficult type of allergic individual in that bacterial studies are usually conducted on the residue cases, the groups which have failed to show evidence of sensitization to the more common food and inhalant allergens. They are the group that Rackemann has very aptly called intrinsic allergics. They are the group that are classed together simply because we have been unable to find an extraneous cause. Some of them may actually be due to extrinsic causes which we have failed to find and the mere absence of an extrinsic cause does not prove that they are all bacterial.

Furthermore this group of intrinsic cases, particularly those of asthma and vasomotor rhinitis, usually have quite a variety of associated nonspecific changes; bronchiectasis, emphysema; polyps and the like which complicate the picture. Even the associated bronchitis and sinus infection may act nonspecifically whether or not there be a specific sensitization to the causative bacteria. In my experience it is not the percentage of satisfactory results with vaccines so much as the occasional startlingly good results in the individual case that leads one to feel that there is a basis for bacterial allergy.

Best results appear to be obtained when the injection of a vaccine produces a local subcutaneous reaction. This has been cited as evidence that the response may be nonspecific. Like Rackemann we have observed that certain of the gram-negative organisms give positive reactions to endermal tests so frequently that one begins to question their specificity. But the following case is difficult to explain on either of these tenets.

A young lady was found to give a very strongly positive delayed reaction to an autogenous pure culture of *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*). The following day the inflammatory reaction covered an area roughly three by seven centimeters. This reaction was accompanied by a severe exacerbation of her asthma. The pure culture vaccine was diluted 1000 times and desensitization with this dilution, which failed to give positive intradermal or subcutaneous reactions, was carried on up through the higher concentrations until she finally received the undiluted vaccine without local reaction and without asthma. In this case the same procedure for desensitization was followed as is employed in pollen desensitization. No local reaction was produced, and the patient was relieved of her asthma.

The exacerbation of attacks of asthma following injections of autogenous vaccine might be interpreted as evidence for specificity were it not that in a given case different vaccines, even though giving negligible endermic reactions will do the same. The exacerbation appears to be a factor of the patient rather than of the vaccine.

With occasional exceptions such as that just cited, my own experience is in agreement with that of Rackemann and of Thomas, that better results are to be anticipated if treatment results in a low grade local subcutaneous reaction. In those cases in which this is requisite the question is again raised as to the specificity of the response.

In this connection the experiments of Mackenzie and Fruhbauer[7] are of interest. They immunized rabbits against egg white until the serum precipitin titer was high. After several months the titer of this egg white precipitin had fallen almost to zero. At this time the injection of typhoid vaccine caused a reappearance of the egg white precipitin which again increased to a high titer. We must infer from these experiments that the injection of a new, foreign, nonspecific antigen will increase the production of other different but specific antibodies. This observation gives us an intelligible clue in our attempt to explain the improvement following nonspecific protein therapy.

Even in those cases such as the one cited above, which improve although there is no local subcutaneous reaction, we cannot prove from present knowledge that the results are not due to a similar type of response.

ARTHRITIS

The question of bacterial allergy is still further complicated by the altogether different type of response to vaccine treatment in chronic arthritis. Swift and his collaborators[11] have produced strong evidence that the joint manifestations of rheumatic fever are essentially allergic and the work of other investigators particularly Crowe,[2] Small[10] and Freiberg and Dorst[3] point strongly to an allergic joint factor in chronic arthritis. My own study of chronic arthritis has followed the general line developed by Crowe.

Our desensitizing vaccine has usually been derived from the colon and in different cases has been either Crowe's stock organisms (*Streptococcus arthriticus* and *Micrococcus deformans*) or an autogenous enteropathogen made up following the Solis-Cohen selective pathogen technic. In an analysis of 100 cases carefully followed over a three year period with vaccine treatment alone we found that 10 per cent had experienced complete remission of symptoms, 37 per cent had obtained a measure of relief which was considered satisfactory, 25 per cent showed some improvement, not enough to be considered satisfactory and the balance remained unimproved. Forty-seven per cent therefore received a satisfactory measure of relief during the period of observation.

The observations of this series that are of present interest and are in contrast to those which we have discussed with the more obvious clinical allergies, are as follows:

In those cases where we have made skin tests with the vaccines, we usually did not observe significant positive reactions either immediate or delayed and no reactions that were of definite prognostic value.

Successful therapeutic results depend upon the complete avoidance of reactions, either local, focal or general. The occurrence of a focal reaction after a vaccine treatment is interpreted as indicating that we are dealing with an organism of etiologic significance but for amelioration of symptoms the dosage must be cut down to where the patient feels better rather than worse after treatment.

In other words, the delayed tuberculin type of reaction appears to be less important, and relief of symptoms appears to depend upon a method of treatment more closely akin to the method of desensitization in experimental anaphylaxis. The evidence suggests that in arthritis associated with bacterial allergy we are dealing with a condition more nearly identical with experimental anaphylaxis, one in which the "third substance" responsible for the tuberculin type of reaction plays much less of a part. If this be true one would expect to find antibodies in the circulating blood. I am not acquainted with any work that has been done on the identication of specific precipitins in the blood of arthritis

but if the work of Hadjopoulos[4] and Burbank on complement-fixation tests for streptococci in arthritics is confirmed, this will give some substantiating evidence.*

In mentioning the need for extremely small dosage of pathogen in the treatment of arthritis, I would emphasize that in a measure, poor results from vaccine treatment of various diseases has been due to failure to recognize the difference between immunization and desensitization. When we are immunizing against a bacterium which may at some time in the future enter the body, experience has shown that large and increasing doses produce best results. Typhoid vaccine serves as an example. When on the contrary the organism has already invaded the system and the host is already sensitized, minute and carefully graded dosage is requisite.

CONCLUSIONS

It becomes apparent from the preceding discussion that our knowledge of clinical bacterial allergy and vaccine treatment is still quite obscure on many points. The factors which enter into the reaction are certainly more complex than those of pollen or other inhalant allergy apparently due primarily to the fact that while with the latter we are dealing with an extrinsic allergen which is absorbed into the system only on occasion, with the former we are dealing with an intrinsic allergen which is constantly present. It appears to be its presence and activity in the body that is responsible for the tuberculin type of reaction, a phenomenon whose explanation will help much in our understanding of bacterial allergy. We are dealing not alone with an antigen-antibody reaction but also with a concomitant specific infection. Successful treatment must therefore do more than merely desensitize against the specific allergen but must also in some way eradicate the focus of living organisms which are responsible for the continuation of the reaction between themselves and the tissues of the host.

The evidence to date indicates that bacterial desensitization is a

* The recent work of Nicholls and Stainsby on agglutinins in chronic arthritis gives most important additional evidence in this regard.

rational procedure and therapeutic results in appropriate cases while not spectacular, justify the procedure. We cannot however hope for any outstanding advances until after the problems just discussed have received further elucidation.

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ACTIVE IMMUNIZATION METHODS AGAINST ACUTE DIFFUSE PERITONITIS*

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Active peritoneal immunization against a possible inflammatory process of the peritoneum is definitely established both by experimental procedure and clinical application.[12, 3, 15, 4, 7, 9] There is a concurrence of experimental data that peritoneal protection can be achieved. As is true with active immunization in general, there is lacking an efficient antigen and further knowledge of some of the basic principles. The mechanism of peritoneal immunity is apparently still controversial. There is disagreement as to whether the immunity is local as understood by Besredka (Herrmann[4]) or is merely a local manifestation of a general immunity (Steinberg and Snyder[16]). There is also doubt as to the specificity of such an immunization. However, there are sufficient data to indicate that the process is not specific. Goldblatt and I[15] immunized animals with colon bacilli and induced fecal peritonitis (the feces contained several species of organisms) with a consequent survival of the animals. Morton[5] used nonspecific substances and secured a peritoneal immunity against a hemolytic streptococcus. It is questionable, however, if Morton obtained invariably a peritonitis in his experimental animals. As I[11, 13, 14] repeatedly pointed out, introduction of bacterial cultures intraperitoneally may not produce peritonitis. The bacteria pass rapidly from the peritoneal cavity into the circulation and the animal may die or survive depending upon the virulence of the organism, but there may be no evidence of peritoneal inflammation. Employment of such a method to induce experimental peritonitis may lead investigators to erroneous conclusions.

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If the peritoneal immunization is of a nonspecific nature, the employment of a single good antigen is preferable, since it lends itself to a more ready standardization. Herrmann[4] failed to secure as good results with *Escherichia coli* (*B. coli*) alone as with a mixture of *Esch. coli* and streptococcus vaccine. My[9] results were contrary to his. Probably, the colon bacillus that I use (our type number 300) happens to be of a better antigenic value.

The rapidity with which the peritoneum can be protected is of significance to the clinician. The quicker the immunity is produced, the more applicable can the procedure be made for the patient. Goldblatt and I,[3, 15] found in our early experiments that an adequate immunity could be established ten to fifteen days after the last immunizing dose. Later, the time interval was shortened and it was disclosed that survival of the experimental animals with an otherwise lethal peritonitis could be accomplished on the same or the following day after the last immunizing dose.[9] Four injections of heat-killed bacteria intraperitoneally on successive days resulted in a large percentage of survivals. Even a single injection induced a protection in some animals. A study of the peritoneal bacterial and cellular reactions led me to conclude that the protection obtained was due to a coincident presence of polymorphonuclear phagocytes in the peritoneal cavity.[10] The phagocytes persisted in the peritoneal cavity for twenty-six days. Previous experiments[8] showed that two factors were responsible for the survival of the animal with peritonitis: (1) rapid phagocytosis and (2) a sufficiently large number of polymorphonuclears to cope with the invading bacteria. The availability of the phagocytes in intraperitoneal immunization, in view of the above conclusions, is probably an important factor in the survival of the animal when the peritonitis is induced several days after the introduction of the vaccine.

It may be stated that the degree of active immunity achieved, with other factors being constant, varies with the antigenic potency of the vaccine. It is fairly generally accepted that living bacteria constitute the best antigen while heat-killed organisms make the poorest. It becomes evident that an incorrect picture may be obtained in the experimental determination of the other

factors (route of vaccine administration, type of organism used, time interval between vaccination and onset of disease) involved in active immunization if the vaccine is of poor antigenic value. Appreciation of the relative inefficiency of heat-killed bacterial vaccine prompted investigators to employ living organisms. Castellani,[2] Pescarolo and Quadrone[6] used living or attenuated typhoid and paratyphoid bacilli for active immunization against the respective diseases. Besredka[1] introduced sensitized vaccine which is a bacterial culture treated first with an homologous immune serum and later killed by chemicals.

TABLE 1

FACTORS CONCERNED IN THE ESTABLISHMENT OF PERITONEAL PROTECTION AGAINST EXPERIMENTAL ACUTE DIFFUSE PERITONITIS

1. THE INDIVIDUAL ANIMAL, ITS CAPACITY TO PRODUCE ANTIBODIES
   a. Quantitative factor (amount of antibody)
   b. Time factor (the rapidity of production of antibodies)
2. THE ANTIGEN
   a. Quality of antigen (measured in degree of antibody response and survival of animal following infection)
3. THE TIME INTERVAL BETWEEN INTRODUCTION OF ANTIGEN AND ONSET OF INFECTION
4. THE SEVERITY OF INFECTION
   a. Qualitative factor (virulence of organism and presence of toxin)
   b. Quantitative factor (the number of organisms introduced at a given time)

The experimental evaluation of a method of peritoneal protection was found to be dependent upon several factors. The capacity and the rapidity of the individual animal to produce antibodies varied with some unknown inherent cause or with some previous antibody stimulating disease (see table 1). It was possible to gauge approximately this variable individual antibody response by a single intraperitoneal injection of a standardized colon bacillus vaccine and hourly peritoneal leukocyte and bacterial counts for twenty-four hours. The severity of the experimentally produced peritonitis could be made constant. Three billion bacteria of a twenty-four-hour plain agar culture of colon bacillus (culture No. 300) suspended in 2.5 per cent gum

tragacanth in saline constituted the fixed peritonitis producing material. In one set of animals, the peritonitis producing material consisted of 5 grams of dog feces from the small and large bowel, suspended in 40 cc. of saline. Such material could be standardized for one series of animals only by using for each animal of that series the material from the same mixture. The amount of the antigen was constant and in the first set of experiments only the quality of the antigen varied. Living and heat-killed colon bacilli were used.

IMMUNIZATION WITH LIVING AND HEAT-KILLED COLON BACILLI

In order to test the difference of the antigenic value of heat-killed and living colon bacilli (culture no. 300), two sets of animals

TABLE 2

IMMUNIZATION WITH LIVING AND HEAT KILLED COLON BACILLI FOLLOWED BY COLON BACILLUS-GUM TRAGACANTH PERITONITIS

DOGS	TYPE OF VACCINE	DAYS BETWEEN FIRST IMMUNIZING INJECTION AND PERITONITIS	DOGS SURVIVED	ANIMALS SURVIVING
				per cent
10	Living colon bacilli	10	10	100
10	Heat killed colon bacilli	10	10	100

consisting of ten dogs each were used. One set was immunized on four successive days by the intraperitoneal introduction of one, two, three and four billion living organisms respectively. The other set received heat-killed *Esch. coli* (one hour at 56–60°C.). Ten days after the first immunizing dose, both sets of dogs with five control animals were injected with three billion living bacteria suspended in 40 cc. of 2.5 per cent gum tragacanth. The control animals died within twenty-four hours with a severe hemorrhagic-fibrino-purulent peritonitis. All the animals of both sets survived (table 2).

Two other sets of dogs were employed to test the relative efficacy of living and heat-killed *Esch. coli* in protecting the perito-

neum against fecal peritonitis. One set of twenty animals was immunized intraperitoneally with living colon bacilli, another set of ten animals with heat-killed bacteria, in a manner similar to the first two sets. Ten days after the first immunizing injection, the thirty animals with five control dogs were injected intraperitoneally with a mixture of fecal material in saline as described above. The five control dogs died in from twelve to thirty-six hours with a marked fibrino-purulent peritonitis. One dog out of the twenty immunized with living organisms died. Four out of the ten immunized with heat-killed bacteria died (table 3).

TABLE 3

DIFFERENCE IN THE PROTECTION CONFERRED BY IMMUNIZATION WITH LIVING AND HEAT KILLED COLON BACILLI IN FECAL PERITONITIS

DOGS	TYPE OF VACCINE	DAYS BETWEEN FIRST IMMUNIZING INJECTION AND PERITONITIS	DOGS SURVIVED	ANIMALS SURVIVING
				per cent
20	Living colon bacilli	10	19	95
10	Heat killed colon bacilli	10	4	40

COMMENT

It is apparent that at least in one set of experiments, heat-killed colon bacilli were able to confer immunity against the homologous organism in a degree equal to that achieved by living bacteria. The colon bacillus-gum tragacanth peritonitis which was induced represents an infection several times the lethal amount necessary to kill a dog of 10 kilograms in weight.

The results of the second experiment in which the dogs were given fecal peritonitis may be interpreted as follows: the fecal peritonitis produced was of a more severe grade than the colon bacillus infection and the heat-killed vaccine was not so efficient an antigen as the living bacteria. It may be concluded also that the infection produced and the immunity achieved bear a quantitative relationship to each other. The immune response as repre-

sented by the number of cells and the rapidity with which they appear must be at least equal to the degree of infection. The fecal material injected contained many different bacterial species. Since the immunizing agent consisted of a single organism and the invading bacteria of several, it may be assumed that the immunity developed was at least in greater part of a nonspecific nature.

INTRAPERITONEAL IMMUNIZATION WITH HEAT-KILLED COLON BACILLI AND PRODUCTION OF A COLON BACILLUS PERITONITIS AFTER VARYING INTERVALS

In previous communications,[9, 10] it was pointed out that when peritonitis was produced a day after the intraperitoneal introduction of a bacterial vaccine, the peritoneal protection obtained was due to a coincident presence of phagocytes. The serum and the

TABLE 4

INTRAPERITONEAL IMMUNIZATION WITH HEAT KILLED ESCH. COLI AND PRODUCTION OF ESCH. COLI PERITONITIS AFTER VARYING INTERVALS

DOGS	DAYS AFTER THE LAST IMMUNIZING INJECTION	DOGS SURVIVING
		per cent
28	1	65
10	14	100

peritoneal exudate were found not to contain any humoral antibodies. The following experiment was performed to evaluate, at least in on approximate relative quantitative measure, the part played by the already present peritoneal cellular exudate and the active immune humoral and cellular processes. Thirty-eight dogs were given four intraperitoneal injections of heat-killed colon bacilli on four successive days. In twenty-eight of these animals, a colon bacillus-gum tragacanth peritonitis was produced the day following the last protecting injection. The remaining ten animals were given a similar peritonitis but fourteen days after the first protecting dose. Peritoneal cell counts taken on the fourteenth day after the first vaccine injection revealed the presence of an average number of 116,000 white cells per cubic centimeter in the peritoneal cavity. Of these cells the polymorphonuclears

constituted 72 per cent and monocytes and clasmatocytes the other 28 per cent. Of the twenty-eight dogs there was a survival of 65 per cent of the animals; of the ten dogs 100 per cent survived. Within the limits of this experiment it may be assumed that the active immunity process accounts for 35 per cent of the survivals or if the protective process were considered on the basis of 100 per cent, the active immunity is equivalent to 35 per cent and the coincident phagocytosis by the leukocytes present in the peritoneal cavity is responsible for 65 per cent of the protection conferred (table 4).

RELATIVE PERITONEAL CELL AND BACTERIAL COUNTS IN NORMAL AND PROTECTED ANIMALS WITH COLON BACILLUS PERITONITIS

Since most of the unprotected control dogs died within the first twelve hours following the production of a colon bacillus-gum tragacanth peritonitis, it was assumed that the survival or the death of the animal was decided within those hours. After peritonitis was induced in animals, peritoneal and bacterial counts were done hourly for eleven hours on control dogs, and in vaccinated animals with peritonitis the day following the last vaccinating dose and fourteen days after the first protecting injection. The relative curves of the peritoneal cell counts are shown in the accompanying chart. The cell counts of the control animal were the lowest of the three. The largest number of cells was present in the dog with peritonitis one day following the last protective injection. It is apparent that in the animal with a definitely established immunity, the cellular response is less than in a dog without such an immunity. However, the previous experiment demonstrated that the actively immune animal possesses approximately 35 per cent greater protection. Under the conditions of these experiments, it may be inferred that the difference in the protection is due to the presence of humoral antibodies which are either bactericidal in themselves or assist the cells in bacterial destruction.

The bacterial counts of the peritoneal fluid were performed by the method of dilution and plating. Hence, all the viable bacteria whether free or phagocytosed were accounted for in the counts.

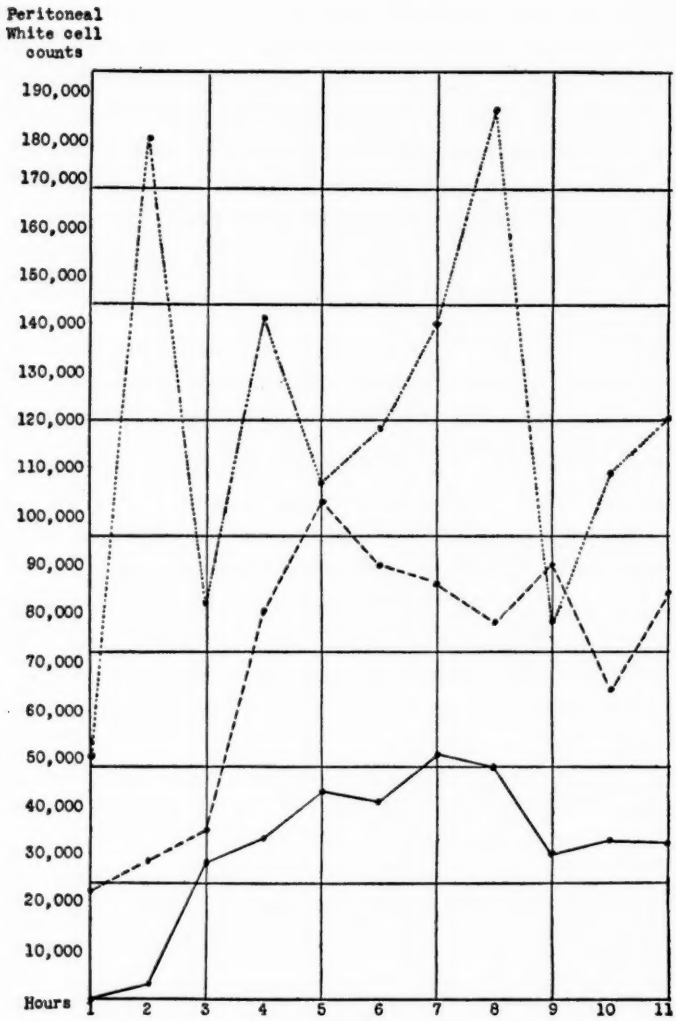

CHART 1. PERITONEAL WHITE CELL COUNTS PER C.M. OF PERITONEAL FLUID

The solid line indicates counts on a normal dog, the line of dashes, a dog with colon bacillus peritonitis fourteen days after the first immunizing injection of vaccine, and the line of dots, a dog with colon bacillus peritonitis on day following the last injection of vaccine.

The relative counts in table 5 reveal that in the control dog with peritonitis, the bacteria persist in large numbers (millions) in the peritoneal exudate; the dog with peritonitis the day following the last vaccination possessed the smallest number of viable bacteria (few thousands). Smears of the peritoneal exudate disclosed that these viable bacteria were within phagocytes. In the animal with a definitely established active immunity, the bacterial destruction was not so rapid nor as thorough as in the dog protected one day. The difference is probably accounted for by the smaller number of

TABLE 5

RELATIVE PERITONEAL BACTERIAL COUNTS IN A CONTROL DOG AND VACCINATED DOGS WITH COLON BACILLUS PERITONITIS

HOURS AFTER ONSET OF PERITONITIS	BACTERIA PER CUBIC CENTIMETER OF PERITONEAL FLUID		
	Control dog	Dog with peritonitis one day following last protecting injection	Dog with peritonitis 14 days after the first protecting injection
1	27,360,000	20,000	18,400,000
2	33,000,000	160,000	2,360,000
3	14,000,000	No growth	400,000
4	6,740,000	No growth	640,000
5	880,000	40,000	480,000
6	240,000	12,600	520,000
7	560,000	9,400	80,000
8	14,000,000	8,400	82,000
9	10,300,000	5,800	20,800
10	4,160 000	4,000	80,400
11	Dead	5,600	15,400

phagocytes in the peritoneal exudate (see chart). These experiments allow the conclusion that the animal protected one day disposes of the offending bacteria more efficiently and rapidly. This bacterial disposal is due entirely to phagocytosis as seen by the absence of demonstrable humoral antibodies and by the observation of phagocytosis in the peritoneal smears. It can be again inferred that other factors than phagocytosis play a part in the protection of the actively immunized animal. It is assumed that these other factors are probably humoral antibodies.

IMMUNIZATION BY THE SUBCUTANEOUS ROUTE

Since the experiments pointed to the establishment of an active immunity, general in character but with a local manifestation it was assumed that subcutaneous immunization should produce at least partial immunity. For the following experiment, two sets of dogs were used. Four daily subcutaneous injections of living *Esch. coli* of one, two, three and four billion organisms were made in a set of six dogs. Another set of nine animals received living *Esch. coli* intraperitoneally on four successive days. Fourteen days after the last immunizing injection both sets of dogs and four control animals were given *Esch. coli*-gum tragacanth peritonitis. The four control dogs died within twelve hours with a severe hemorrhagic-fibrino-purulent peritonitis. The two sets of dogs, six and nine in number, survived. Apparently, the general immunity established by the subcutaneous route was sufficient to protect the animals from an otherwise lethal peritonitis.

SUMMARY

Heat-killed colon bacilli used as intraperitoneal vaccine can induce a protection against a colon bacillus peritonitis equal to that of living bacteria. However, in fecal peritonitis the living colon bacilli confer a greater protection than heat-killed organisms. It is assumed that this difference is due to a greater antigenic value of living bacteria. Because colon bacilli vaccinated animals survive fecal peritonitis, (feces contain many species of bacteria) the immunity is considered, at least in greater part, nonspecific. The protection against acute diffuse peritonitis is of a two-fold nature; a general immunity and a coincident presence of phagocytes in the peritoneal cavity with a consequent phagocytosis. The active general immunity can be evaluated as 35 per cent and the local phagocytosis as 65 per cent under the conditions of these experiments. The greater number of these phagocytes is polymorphonuclear in type. This general immunity is apparently sufficient to protect animals by subcutaneous route against a colon bacillus peritonitis.

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THE RELATIVE VALUE OF CULTURAL METHODS AND GUINEA PIG INOCULATION IN THE DIAGNOSIS OF TUBERCULOSIS*

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In recent years a lengthy discussion has been carried on relative to the value of cultural methods and use of guinea pigs in the diagnosis of tuberculosis. It has led to a rather sharp division of opinion which has obscured the facts and resulted in individuals advocating one method to the exclusion of the other. With a view of carefully appraising the methods, we have reviewed the work of previous authors and have conducted extensive experiments of our own. Advantages and disadvantages have been found in both methods and wise clinical pathologists will make use of each for what it does best. Out of the controversy has certainly come improved and excellent methods for culturing organisms of tuberculosis, stimulated by Löwenstein[23] in Europe and Corper[5] in America. Furthermore, a clearer understanding of the limitations of animal inoculation has been realized, as well as the great value of testing clinical material by inoculation of guinea pigs.

SPONTANEOUS TUBERCULOSIS IN GUINEA PIGS

One of the objections that has been made to the use of guinea pigs in the diagnosis of tuberculosis, is that these animals, since they are susceptible to the disease, may become spontaneously infected, giving rise to a false positive. Robert Koch[22] was the first to issue a caution concerning the interpretation of results in guinea pigs. Although he never had a tuberculous pig brought

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to him for study, he did report on seventeen that contracted tuberculosis while under observation in the laboratory in which other tuberculous animals were kept, and he stated that caution should be exercised in the interpretation of results when guinea pigs are kept for more than three months in the room with tuberculous animals.

Calmette[2] who has had a vast experience with guinea pigs, stated that tuberculosis practically never affects them under ordinary circumstances. This same opinion had previously been voiced by Corbett.[4] Guinea pigs can, however, be readily inoculated by nasal spray, and Römer and Joseph[38] maintained that the guinea pig is so susceptible to tuberculosis that one single bacillus probably can produce tuberculosis in them.

Bartel[1] found that suckling guinea pigs became tuberculous if the mother had the disease. The investigation of Remlinger disclosed that even though guinea pigs were confined with other animals which had cutaneous ulcers due to tuberculosis, very few would develop the disease; thus, of forty-three normal guinea pigs confined with those which had cutaneous ulcers, until the latter died, tuberculosis developed in only three. Fifty-two guinea pigs were fed for a year on material which had been soiled by tuberculous animals, without contracting the disease. Sewall and Lurie[42] confined guinea pigs under small bell jars used for both positive and negative animals, and returned these animals to crowded, unsterilized cages; a third of eighteen studied contracted the disease.

Perla[34, 35] demonstrated that after intraperitoneal inoculation, *Mycobacterium tuberculosis* is eliminated in the feces for the first week, following which none appears for several weeks unless the disease is widely disseminated in the body. In such instances organisms occasionally appear in the bile and in the urine. He quoted Koch to the effect that spontaneous tuberculosis in animals was in direct proportion to the number of animals kept in the room, and that the mode of transmission was respiratory. Perla conducted experiments to demonstrate the effect of overcrowding and the presence of tuberculous cutaneous ulcers and their importance on the development of spontaneous tuberculosis

in guinea pigs. Under the conditions of the experiment a few of these guinea pigs contracted tuberculosis, and when normal pigs were confined in the same cages with infected animals the portal of entry was by the intestinal tract. He also concluded that when guinea pigs were kept in the same room with tuberculous animals even though they were in different cages, after three months they were not suitable for experimental purposes; in such instances the portal of entry was the respiratory tract.

Griffith[17] observed seven cases of spontaneous tuberculosis in guinea pigs, over a period of fifteen years. He stated that in 1928 he had two in which the infection proved to be avian in type. In 1927 he observed another case due to the human organism of tuberculosis. Unfortunately, these animals had been used previously for injection of tuberculous material, and this would cast some doubt on the reliability of the observation.

Reisman and Baylis[37] performed, the following experiment: twelve young, presumably normal guinea pigs were allowed to remain for four days in an enclosure on the floor of the animal room; then, a second lot of animals was placed in the same enclosure. At the end of the period, 75 per cent of the first lot gave positive skin reactions with tuberculosis and 25 per cent of the second. Some of the guinea pigs were subjected to necropsy; in only two were acid-fast bacilli found, but these organisms were not tested for virulence. The authors concluded that tuberculosis is quite prevalent in apparently normal guinea pigs. The conclusion is especially interesting in view of the work by Thompson and Frobisher,[47] who quoted Corper and Petroff to the effect that they found acid-fast bacilli in 36 per cent of a large series of animals previously given injections of filtered tuberculous material, but that they also demonstrated such organisms in 33 per cent of normal animals which had not been given injections. Thompson and Frobisher performed a similar experiment with similar results, and stated that all observers failed to culture or identify the organism; hence, they felt that the presence of acid-fast bacilli in the lymph nodes of guinea pigs should not be taken as an indication of tuberculosis.

Sewall[41] is of the opinion that guinea pigs kept in sanitariums for

tuberculosis should be very carefully guarded against being given scraps from the tables of patients and protected from contact with caretakers who might have tuberculosis. Lurie,[24, 25, 26, 27] in a series of experiments on the experimental epidemiology of tuberculosis, has concluded that the more guinea pigs are crowded the more spontaneous tuberculosis appears in them. However, as an index of air-borne infection, he was able to keep twelve controls in the room, not in contact with the others,—negative during the year of observation. In a room in which the number of tuberculous guinea pigs varied, out of 103 which originally were free of tuberculosis and which were exposed for thirty-two months, 14.5 per cent developed tuberculosis; obviously the respiratory tract was the portal. Even when normal guinea pigs were exposed in the same cages with others which had tuberculosis, and the cages were made with wire mesh bottoms, practically all of the evidence pointed to the fact that the infection was pulmonary, and was not developed until after an average of 293 days. In an experiment he controlled conditions to some extent, so that guinea pigs could acquire tuberculosis either by the respiratory or the digestive tract. He was able to show that when guinea pigs acquired tuberculosis naturally in this fashion, the infection did not become disseminated, but remained near the portal of entry.

In order to test to some extent the question of spontaneous tuberculosis occurring in guinea pigs, a series of observations and experiments was made in our laboratory. Guinea pigs have been used as experimental animals in The Mayo Clinic for about twenty-seven years; during this time thousands of these animals have been subjected to necropsy. Although specific search for tuberculosis has not been made in all cases, nevertheless records are available which include more than 25,000 postmortem examinations of guinea pigs which have been received from scores of different dealers in the United States, in particular from those in the middle west. In no instance has spontaneous tuberculosis been encountered in one of these animals. In addition to this evidence, one of us (T. B. M.) has given injections to more than 15,000 guinea pigs for diagnosis of tuberculosis, all of which have been subjected to careful necropsy. Approximately 15 per cent

of these animals has been positive, which means that a positive diagnosis has been made on about 1,200 specimens by this method. In most instances it has been possible to confirm the findings by operation on, extensive clinical observation of, or necropsy of the patient, and in not a single instance has a false positive been reported, which indicates even more strikingly the fact that spontaneous tuberculosis in animals which have been brought to the laboratory for use has not occurred in our series.

However, the question of whether animals can contract tuberculosis when kept in the same room or the same cages with animals which have tuberculosis is quite a different problem, and has an important bearing on how animals are to be cared for during experimental work. Accordingly, a series of experiments was designed to test the development of spontaneous tuberculosis in guinea pigs in our laboratories under certain conditions.

The animal room, which is used exclusively for housing animals that are to be used in tests for tuberculosis, is 20 feet wide and 40 feet long. Cages are arranged in four groups, with forty-eight cages in each group. The individual cages, of all-metal construction, are designed to house two guinea pigs each, and to allow 108 square inches to the pig. There is provided a steam sterilizer for sterilizing the cages after each pair of guinea pigs has occupied it.

Four cages were selected, one from each group of cages, so chosen that two were on the bottom row and two on the top row. Into each of these cages two normal guinea pigs were introduced. They were cared for by the same caretaker who handled the rest of the animals in the room.

In the first cage one animal died at the end of fifteen days, and the other one was killed at the end of 396 days. Neither animal showed any signs of tuberculosis. In the second cage, one animal lived 137 days and the other was killed at the end of 396 days. Neither animal showed any signs of tuberculosis. In the third cage one animal died at the end of thirty-four days, the other one at the end of seventy days, neither one showing any signs of tuberculosis. In the fourth cage, which was one on the bottom row of cages, one animal was killed at the end of 396 days, showing no signs of tuberculosis, whereas the other animal of the pair died in 221 days with typical tuberculous lesions in the liver, spleen, lungs and bronchial lymph nodes.

For other experiments special cages were designed, of such size that each guinea pig was allowed fifty-two square inches of space. These cages were open at the top and were arranged in the following fashion: Cage 1, beginning on the left, was a cage in which were placed ten normal guinea pigs. Cage 2 was separated from number 1 by half inch mesh wire screen, so that it was possible

for portions of food and bedding to be interchanged between the cages by the antics of the guinea pigs. In this cage were placed ten guinea pigs, five of which had been given intraperitoneal injection, and five subcutaneous injection of an emulsion of tuberculous spleen, freshly removed under precautions of sterility, from a guinea pig. In this same cage were placed fifteen normal guinea pigs. Cages 3 and 4 were duplicates of cages 1 and 2, both in size and in the manner in which the experiments were carried out. The same person who took care of the other guinea pigs in the room took care of cages 2 and 4, whereas another person, who had nothing to do with tuberculous animals, took care of cages 1 and 3. The cages were cleared of bedding once a week and only one supply of water and one hopper of dry food was kept in each cage.

Of the guinea pigs in cage 1, four were dead by the end of thirty-eight days; the other six lived from fifty-six to 396 days. None of these animals showed evidence of tuberculosis; apparently they died of various other causes. Of the guinea pigs in cage 2, that received injections of tuberculous material, five died within the first nineteen days and showed no signs of tuberculosis. The other five lived for from forty to eighty-six days, and all had typical miliary tuberculosis. In that cage of the pigs which were not inoculated, eight died within thirty-three days, seven lived for from forty to 396 days; none showed any signs of tuberculosis. In the third cage, which contained pigs which were not inoculated, four died within thirty-seven days; the remaining six lived for from 186 to 396 days and none showed any signs of tuberculosis. Of the inoculated pigs which were kept in cage 4, four died within fourteen days without signs of tuberculosis. The remaining six lived for from thirty-two to 134 days, and showed evidence of typical miliary tuberculosis. Of the fifteen pigs which were not inoculated but which were housed in the same cage, eight died within thirty-two days and the remaining seven died in from ninety-two to 396 days; all of this lot were negative for tuberculosis except one pig, which lived 179 days, and which, at necropsy, revealed a typical tuberculous spleen.

It is interesting to note that four young guinea pigs were born to mothers in the second cage; one of them died 240 days later, two were killed 300 days later, and a fourth died 330 days later. Three additional animals were born in the same cage and lived about ninety days each. None of these members of the second generation showed any signs of tuberculosis.

From those observations and experiments, and from the observations of others, it becomes evident that when guinea pigs are kept in cages in a room free from tuberculous animals, and receive no food from the tables of tuberculous patients, and are not cared for by tuberculous caretakers, the chances of their becoming infected with tuberculosis may be totally ignored, and any such animal, provided it is in normal health, may be considered suitable for experiments on tuberculosis.

It is possible for animals, especially if they are over-crowded when kept in the same room with tuberculous guinea pigs or other tuberculous animals to contract spontaneous tuberculosis. Under the worst conditions, in only a relatively few animals will such lesions develop and these will not develop in less than ninety days and usually a longer time will be required. When such lesions occur they are usually discrete and can usually be distinguished from the lesions produced by experimental inoculation of the animals, as has been shown by numerous workers. It is evident that under most conditions, by use of very simple precautions, stock guinea pigs could be kept in the same room with a few animals which were used for the diagnosis of tuberculosis. If one were using only a few such animals, very little exposure would ever be brought to the normal stock guinea pig. If many animals are used, such a laboratory would undoubtedly have available a stock-room which would be different from the room in which the diagnostic pigs were kept, and under these conditions spontaneous tuberculosis in experimental guinea pigs would be effectively ruled out.

CULTURE METHODS FOR MYCOBACTERIUM TUBERCULOSIS

It is not the purpose of this paper to review in detail the various methods which have been proposed for culturing *Mycobacterium tuberculosis*. However, it is desirable to give a résumé of the important steps which have been taken in bringing this useful laboratory method to its present state. Robert Koch[22] successfully grew bacilli of tuberculosis in 1882, using coagulated blood serum derived from tuberculous tissue. While some improvement was made after that time, it was not until Petroff, in 1915, devised his method that the culturing of bacilli of tuberculosis from clinical material became at all practical. To be sure, Uhlenhuth had introduced antiformin as an anticontaminant reagent, but Petroff introduced 3 per cent sodium hydroxide as a killing agent for nonacid-fast bacteria, which was far superior. He made use of egg medium of Dorset, which had been successfully used by that author in 1902, and added to it besides certain other ingredients and gentian violet to suppress the growth of

gram-positive cocci. Between the time of Koch's and of Petroff's work, Roux and Nocard had discovered the importance of glycerol in mediums used for growing bacilli of tuberculosis. Although Petroff's method has been extremely useful, it has by no means proved a satisfactory medium for the routine culturing of bacilli of tuberculosis, derived from clinical material, and the introduction of the combination of killing nonacid-fast bacilli with sulphuric acid, and the subsequent planting of the sediment on glycerol-potato medium was introduced by Sumiyoshi[44] in February, 1924. This was followed, the next month, by further studies by Löwenstein[23] using this method. Hohn,[20] in 1926, modified the Sumiyoshi-Löwenstein method in two particulars: by the use of the unwashed sediment for culturing, and by the substitution of egg medium for glycerol-potato.

In the hands of these authors and numerous others, these methods have proved practical and successful, and it has been demonstrated beyond any question that positive cultures often can be obtained when specimens are microscopically negative. Corper[5] has estimated that unless 100,000 bacilli are present in 1 cc. of sputum, it is not possible to detect them in the stained smear; however, Pottinger[36] claimed, by his method, to be able to demonstrate bacilli of tuberculosis when they were no more numerous than 250 for each cubic centimeter.

Corper and Uyei[6, 7, 8, 9, 10, 11] have studied the Sumiyoshi-Löwenstein method extensively, and have introduced several modifications; using potato cylinders, first devised by Pawlowsky,[33] they added crystal violet and glycerol water, treating contaminated material with oxalic acid, 5 per cent, which, after a long series of experiments, they concluded was the best method for culturing *Mycobacterium tuberculosis*. So successful were they with this method, that they were able to grow bacilli of tuberculosis from 91.4 per cent of sputums demonstrated microscopically to have bacilli in them. Matthies[29] reported that using Hohn's modification, cultures were obtained in nearly all material which was demonstrated to be microscopically positive.

Sweany and Evanoff[45, 46] have proposed using a medium composed of veal, milk, cream, and egg, using 5 per cent hydrochloric

acid or 3 per cent sodium hydroxide for killing nonacid-fast bacilli. This medium has been slightly modified by Feldman,[14] and has proved extremely satisfactory in isolating bacilli of tuberculosis, and in particular the bovine type. Still more recently, Miraglia[31] has introduced glycerol-broth-egg-yolk medium, which in his hands and in the hands of Feldman,[15] has been extremely satisfactory, in particular for the human type of *Mycobacterium tuberculosis*. Still more recently, Herrold[18] has used egg-yolk in nutrient agar, pouring plates or slants. He concluded that this method was more certain than smears and is as delicate and more prompt than guinea pigs. Strangely enough, however, the author made these sweeping conclusions on the basis of few specimens. There were three specimens of urine, two of which were shown to contain bacilli of tuberculosis by smears, and all gave growth in from seven to ten days; three specimens of sputum all of which were shown to contain bacilli of tuberculosis by smear, gave growth at the end of ten days; one specimen of spinal fluid, the smears of which were negative, gave growth on the tenth day, and two specimens of thoracic fluid, smears of which were negative, gave colonies in six days. So far as can be determined from Herrold's paper, guinea pigs were used only in a dilution experiment, in which five guinea pigs were used for comparison of results with those obtained by examination of smears and by culture. The guinea pigs and the cultures ran parallel; the smears became negative in the high dilutions.

As the matter now stands, it may be conceded without any question that bacilli of tuberculosis can be cultivated readily in a high percentage of instances when they can be found in direct smears, and that colonies will appear so that they can be detected after a period of ten days or more, average two to five weeks; the time depends somewhat on the medium used, the method of destroying contaminants, the type and number of bacilli in the material. As compared with the method of direct staining, it is evidently more sensitive, but enough evidence is not available to give a fair estimate as to how much more sensitive it actually is, and indeed the problem hardly seems to warrant the time and money it would take to solve it.

Although it is one thing to demonstrate the presence of bacilli of tuberculosis by cultural methods in material which on microscopic examination can be shown to contain these organisms, it is quite another to culture organisms from material which is microscopically negative. Since from a diagnostic standpoint this is the most important phase of the study, we have made a thorough comparison between the results of such cultures and inoculations of guinea pigs.

CULTURES COMPARED WITH ANIMAL INOCULATION

Löwenstein[23] studied fourteen specimens of urinary sediment, which were cultured and inoculated into guinea pigs. Tuberculosis in three animals failed to develop, but the cultures were positive. Löwenstein did not present any evidence to indicate whether these cultures were of virulent organisms, or whether the patients from whom this material was collected had tuberculosis.

Clairmont[3] found in the examination of clinical material one instance in which culture was positive and the animal negative, whereas three times the animal was positive and the culture negative. Information is not available as to the exact interpretation of these results.

Seelemann and Klingmüller,[40] as a result of experiences with Hohn's procedure, came to the conclusion that inoculation of animals is to be preferred on account of its greater certainty, especially when the material is contaminated with spore-forming microörganisms.

Corper,[5] and Corper and Uyei,[8] who advocated the cultural method as a certified diagnosis for tuberculosis, came to the conclusion that this method is equal to inoculation of guinea pigs and has many advantages. The basis on which this conclusion is drawn, however, will bear some examination. For instance, they studied ninety-three microscopically positive specimens of sputum. They obtained positive cultures in 91.4 per cent, but made the following interesting and suggestive statement: "To have done guinea pig inoculation tests in this series would obviously have been unnecessary so they were omitted." In a series of nine

microscopically doubtful specimens of sputum, the guinea pig and culture method ran parallel. Of twenty-four specimens of urine these investigators used, three gave positive cultures by Petroff's method, ten gave growth in potato medium, and eleven gave positive results with guinea pigs. The tissues from dogs and rabbits infected with tuberculosis, when cultured and also used for inoculation of guinea pigs, gave results about parallel, but the nature of the experiment is such that it is difficult to point out advantages for either method. The same experimenters stated that the cultural method would usually give positive results in the fourth to the fifth week, and guinea pigs in the third to the fifth week. They further stated that there is no difficulty in determining pathogenic from nonpathogenic acid-fast bacilli, since the usual nonpathogens will grow on the ordinary culture mediums in from two to five days. Apparently they did not do virulence tests on the organisms isolated, nor did they correlate any of their results with the condition of the patients.

Corper and Uyei,[11] in comparing various methods of growing bacilli of tuberculosis, have reported in their tables on the basis of the number of tubes inoculated, from which they obtained percentages of positive growth. Interpreting these results on the basis of clinical specimens is not altogether obvious, unless one has available the exact protocols of each group of cultures. Very different percentages might be obtained on the basis of the number of specimens used from those on the basis of the number of tubes used.

The difficulty of obtaining growth of bacilli of bovine tuberculosis on mediums which have been devised by Corper has been reported by Feldman,[15] who was able to show that the medium of Sweany and Evanoff was superior in this regard. Even Corper and Uyei[11] had difficulty in obtaining a large percentage of positive cultures with bovine material when compared with the percentage of positive results obtained with inoculation of guinea pigs. Thus, of sixteen specimens used, nine proved positive by cultural methods, whereas all specimens were positive, judged by results of inoculation of guinea pigs. The importance of this in relation to the diagnosis of tuberculosis in man is obvious, when

one recalls the rather high percentage of bovine tuberculosis in human beings that has been reported from time to time by various authors, and the more recent report of Van Es and Martin,[48] who obtained bovine tuberculosis in several specimens derived from human beings, including those sent to them from The Mayo Clinic.

Herrold's[18] work has been considered elsewhere in the paper; suffice it to say that on a basis of very little evidence he concluded that cultures were more delicate and results of cultures more prompt than inoculation of guinea pigs. Nevertheless, Woolsey,[49] has submitted the medium to a more critical test. She made a routine of testing suspicious fluids, both by this cultural method, slightly modified, and by inoculation of guinea pigs. Of 130 cases, in twenty-five cultures were positive and in twenty-three results with guinea pigs were positive; there was agreement in 121 instances. The cultures became positive, on an average, in eleven days, whereas the diagnosis by inoculation of guinea pigs was not made until six weeks had elapsed. As Woolsey became more proficient with the medium, her cultures became positive, on an average, in five days; some in as short a time as two days. In her experiments with animals she used the inguinal nodes, making smears and sections of them, without depending on gross characteristics. Of five of the nine cases in which the results disagreed she concluded that the Herrold medium was the more reliable. Three of her cultures became over grown and had to be discarded. She injected the isolated bacteria into guinea pigs when there was disagreement between the results of culture and the original inoculation of guinea pigs. By the use of guinea pigs, three cases were detected in which the cultures were negative, whereas by the use of cultures four cases were identified in which the guinea pigs were negative.

Húth and Lieberthal,[21] reporting on 1,200 cultures made by Hohn's method, stated that this method is to be preferred to the use of guinea pigs. Protocols were omitted from the paper, and it is evident that the same material was cultured and also inoculated into guinea pigs in only "about ten cases;" yet the authors used the expression "in many cases in this latter group" referring

to the cases in which guinea pigs were inoculated, and they further stated that: "because of its absolute reliability and simplicity the culture method is of greater value than the guinea pig inoculation." From the material they presented, it is impossible to know on what basis these conclusions were drawn.

Schmidt[39] examined forty specimens of sputum in which bacilli of tuberculosis were scantily present. When cultured, they gave, with the egg medium, six positives, and with the potato medium, five, whereas eleven guinea pigs gave positive results. Schmidt concluded that the animal test was more sensitive than the culture method. In one case, however, the animal test was negative and the culture positive, so that he recommended using both methods.

Dimtza,[12] studying specimens obtained at surgical operation, compared the results of culturing with inoculation of guinea pigs. He used a slight modification of Hohn's method but did not give the method used in handling the animals. He examined 500 specimens, of which he concluded 126 were tuberculous. Of these, 104 (83 per cent) proved to be positive by direct smear, 117 (93 per cent) were demonstrated to be positive by inoculation of animals, and 122 (97 per cent) were positive by culture. In one case direct smear was positive, and the other two methods gave negative results. In three cases both smear and culture were negative, and in eight both smear and inoculation of guinea pigs gave negative results. No evidence was presented to prove that the eight positive cultures were actually *Mycobacterium tuberculosis*, since he apparently did not test them in animals. Obviously only eleven specimens that were microscopically negative were tested, and, lacking the protocols of the eight negative animals, final conclusions are not evident.

Recommendations similar to those of Schmidt were made by Sweany and Evanoff.[46] They found agreement in direct smears, cultures, and inoculations of animals in study of ten specimens of material suspected of being tuberculous, and in which all three methods gave negative results. With fourteen specimens the following results were obtained: three specimens were positive by direct smear, culture, and animal inoculation; the other eleven

were negative by direct smear. Of these eleven, nine were positive both by culture and inoculation of animals. Two were negative by cultural methods and positive by inoculation of animals, and three were negative by inoculation of animals and positive by culture. These three cultures were injected into guinea pigs and proved to be bacilli of tuberculosis. The number of animals given injections of each specimen was not given, but the authors stated that there were 11.7 per cent more positive results on culture than on inoculation of animals. It is not at all evident how these figures were obtained, since out of a total of fourteen specimens, eleven were positive by animal inoculation and twelve were positive by cultural methods. The authors concluded that for diagnostic work both methods should be used.

Stadnichenko and Sweany[43] later compared inoculation of guinea pigs and cultures in a series of 200 specimens of sputum of selected patients. One hundred thirty-one patients were found to be negative as judged both by results of inoculation of guinea pigs and of culture; thirty-three were found to be positive as judged by results of both methods. In thirty-three cases inoculation of guinea pigs gave positive results whereas the cultures were negative, and in three cases the cultures were positive whereas guinea pigs were negative. Two of these cultures were tested on animals and proved to be virulent. They concluded that inoculation of guinea pigs was superior to the cultural method for badly contaminated material such as sputum. Cultural studies had the advantage of being more absolute, less expensive and of permitting of more repetition. The authors voiced a warning that atypical acid-fast bacilli should be submitted to animal experimentation before a positive diagnosis was given. They also expressed the belief that certain specimens will produce growth only when treated by acid; others, only when treated by alkali.

Woolsey and Petrik[50] compared the results of inoculation of guinea pigs with cultures after having devised a potato-egg medium which they claimed to be superior both to Petroff's and to Corper and Uyei's medium. The material tested was specimens of sputum from patients either known to have tuberculosis or

suspected of having it, and in which only a few bacilli, or none at all, could be found. They concluded that acid digestion of the sputum resulted in fewer contaminations, but that alkalies made better agents of digestion. Comparing the results of inoculation of guinea pigs with results with their own medium it became quite evident that a higher percentage of positive tests could be obtained by use of guinea pigs than could be obtained by culturing. The authors pointed out that one can inoculate guinea pigs with larger amounts than it is practical to use in cultures, and that this often accounts for the higher percentage of positive results with guinea pigs. Trying this out on a quantitative basis they concluded that at least twenty tubes of this medium would have to be inoculated in order to equal the accuracy of inoculation of guinea pigs when the animal receives a considerable amount of material. Nevertheless, they claimed that positive results can be obtained more readily and earlier by the cultural method. The earliest growth was obtained after eleven days, and the latest after fifty-six days, with an average of twenty-one days. Fifteen per cent of all tubes were contaminated, but no set of tubes was completely contaminated. The guinea pigs were tested with tuberculin once a week, and as soon as this test was positive, they were killed. The earliest was killed in eighteen days after inoculation; the latest, in sixty-eight days, and the average, in thirty days. No animals were called positive unless the organisms were demonstrated in the lymph nodes.

In a personal communication from Hillkowitz,[19] he stated that Puntoni summed up the whole matter as follows: "The cultural method, according to the most recent research, cannot presume to be a substitute for the guinea pig inoculation, which always gives the greatest number of positives. Nevertheless, it presents certain advantages especially speed and occasionally positive results when the biologic test fails."

As a result of about fifteen years' experience with inoculation of guinea pigs for diagnosis of tuberculosis, and on the basis of carefully controlled tests, we[16] have found that the safest procedure for inoculation of animals with clinical material is as follows: the material is centrifuged for one hour at 2,500 revolutions per

minute, in a centrifuge that has a head radium of 13.5 cm. At the end of this time the top 1 cc. is pipetted off, and after the middle portion has been discarded, this top 1 cc. is added to the sediment. The whole is now made up to 5 cc. with physiologic saline solution, and two guinea pigs are given injections, one subcutaneously and one intraperitoneally. Guinea pigs which die and show no gross lesions by the end of three weeks are considered failures; if no lesions are present in guinea pigs that die after three weeks, the test is considered negative. Guinea pigs are premitted to live for eight weeks; at the end of that time they are killed, and considered either positive or negative, depending on whether lesions are present or absent. To anyone experienced in the diagnosis of tuberculosis of such animals, gross lesions can be accurately diagnosed without resorting to histologic examination of the tissue. In certain rare cases, especially when the animal has died early, and histologic diagnosis of tuberculosis must be used, it should be based primarily on finding acid-fast bacilli within the lesions.

In an attempt to hasten the diagnosis of tuberculosis in guinea pigs, we[28] experimented with intracerebral inoculation of animals with clinical material. This proved to be unsatisfactory as a routine, on account of the large number of failures among the inoculated animals. In certain instances, however, the diagnosis of tuberculosis in these animals could be made as early as nine days after inoculation.

Some workers have increased the speed of the test by submitting guinea pigs, at the end of three or four weeks, to a test with tuberculin, killing those that give a positive reaction. Our experience with this procedure is too limited to allow a definite opinion to be expressed, but evidence would point to the fact that in the majority of cases this procedure is safe.

EXPERIMENTAL

In comparing results of cultures of clinical material with inoculations of guinea pigs, approximately one-third of the material was seeded in not less than eight tubes of culture mediums, whereas the rest of it was inoculated into a pair of guinea pigs, and almost always the animals were allowed to live until they died, or at least fifty-eight days. The mediums used were those proposed by

Corper and Uyei, Sweany and Evanoff, and Miraglia. Not all of the specimens were cultured by all three methods, but most of them were. In addition, both

TABLE 1

DAYS ELAPSING BEFORE INOCULATED GUINEA PIGS, AND CULTURES BECAME POSITIVE

(All material microscopically positive)

URINE (21 SPECIMENS)		SPUTUM (10 SPECIMENS)		JOINTS, TISSUES, ETC. (6 SPECIMENS)	
Guinea pigs	Cultures	Guinea pigs	Cultures	Guinea pigs	Cultures
59	45*†	48	17‡	20	63§
27	32§	61	16‡	36	53*
55	48§	48	14‡	62	28*
42	37*	29	20§	42	13§
51	60§	62	10§	22	25*‡
57	30*§	60	15‡	62	13*‡§
56	28†	40	16‡§		
63	35*	61	16‡		
35	37*	60	15‡		
59	56§	65	21§		
67	64§				
37	19‡				
43	14‡				
32	18‡				
42	36§				
51	32*				
47	46‡				
40	Negative	Tuberculosis, bilateral renal (clinical), pulmonary (clinical), knee (surgical pathology)			
Failure	Negative	Tuberculosis, left renal (surgical pathology), pulmonary (clinical)			
Failure	Negative	Tuberculosis, left renal (surgical pathology)			
Negative	Negative	Tuberculosis, left renal (surgical pathology)			
Average..48	38	53	16	41	33

* Glycerol potato (crystal violet).
† Glycerol potato (without crystal violet).
‡ Glycerol-broth-egg-yolk.
§ Veal-milk-cream-egg.

acid and alkali were used as an anticontaminant reagent. Results of the first series of tests are summarized in table 1. All of the material used was demonstrated to contain acid-fast bacilli by direct smear, and the Ziehl-Neelsen stain.

Twenty-one specimens of urine were tested; eighteen produced tuberculosis in guinea pigs, whereas seventeen cultures were positive. Two pairs of guinea pigs died before three weeks had elapsed, and were therefore failures. Cultures from these specimens were negative, and in the remaining case both the cultures and the guinea pigs were negative. The four cases which yielded negative cultures were clearly cases of tuberculosis. The average period of time elapsing before the cultures were positive was thirty-eight days, varying from fourteen to sixty days. The average number of days before the guinea pigs were diagnosed as positive was forty-eight days, varying from twenty-seven to fifty-nine days. Ten specimens of sputum were used, and there was agreement between results of culture and of inoculation of guinea pigs in each instance; all specimens proved to be positive. The average number of days for the cultures to become positive was sixteen, and for the guinea pigs, fifty-three. Six specimens from joints, tissues, and so forth, proved to be positive by inoculation of guinea pigs and culture; the average time for cultures was thirty-three days and for guinea pigs, forty-one days. The greater number of days elapsing before the guinea pigs were positive is partly explained by the fact that the guinea pigs were allowed to live until they died. Diagnosis of many of them could have been made at a much earlier period; one diagnosis was actually made at the end of twenty days.

It becomes evident, therefore, that inoculation of material which is microscopically positive for bacilli of tuberculosis will yield positive cultures in a large percentage of instances, and, by the method of Miraglia, in from ten to thirty days. However, in keeping with the findings of others, in a certain small percentage there is no growth; less often, guinea pigs fail to become tuberculous after injection of such material.

Occasionally, in the routine of work, we have had occasion to inject into guinea pigs material known to contain acid-fast bacilli obtained from lesions which were either then known to be tuberculous or later were demonstrated to be so, and following which tuberculosis failed to develop in the guinea pigs. Two such instances were reported by Morse and Braasch[32] in a series of inoculations of guinea pigs made by one of us (T. B. M.). Although the explanation of this is not altogether apparent, several possibilities suggest themselves: scarcity of bacilli of tuberculosis, the fact that the bacilli may be dead, avirulent or avian, and the fact that bacilli of tuberculosis often appear in clumps, so that those demonstrated on the slide may be the only bacilli present in the material; hence, no organisms might be injected into the

animals. Striking results along this line can be noted in, to get ahead of the story somewhat, table 5; in some animals lesions developed whereas in others none developed, although injections were with material from the same specimen, and were made at the same time.

In order to test the efficacy of cultures as compared with inoculations of guinea pigs, working with clinical material that is negative by direct smear, 100 such specimens, including eighty-four specimens of urine, six of thoracic fluid and ten of material drained from joints, tissues, and so forth, were injected into guinea pigs and cultured simultaneously, using the three methods before indicated.

TABLE 2

MEDIUMS AND ANTICONTAMINANT REAGENTS USED FOR CULTURE OF 100 CLINICAL SPECIMENS MICROSCOPICALLY NEGATIVE*

	CRYSTAL VIOLET-GLYCEROL-POTATO			GLYCEROL-POTATO			VEAL-MILK-CREAM-EGG		
	Specimens	Tubes used	Tubes contaminated	Specimens	Tubes used	Tubes contaminated	Specimens	Tubes used	Tubes contaminated
Sodium hydroxide	17	103	31 (30%)	8	38	15 (39%)	8	63	57 (90%)
Sulphuric acid	26	200	78 (39%)				9	71	36 (50%)
Oxalic acid	22	158	8 (5%)	10	75	10 (13%)	18	136	81 (60%)

* Seventeen specimens with all tubes inoculated, contaminated.

Table 2 indicates the results in respect to the contaminations in the cultures, using different methods, and it can be readily seen that oxalic acid combined with crystal violet glycerol-potato medium gives the most satisfactory results. However, it should be kept in mind that this method does not yield the highest number of positive cultures.

Tables 3 and 4 give a comparison of the results obtained. It will be seen that nine specimens of urine were positive as judged by inoculation of guinea pigs, and only two by cultural methods. Two specimens of thoracic fluid were positive by inoculation of guinea pigs whereas none was positive by cultural methods.

Three specimens of material derived from joints, tissues, and so forth, were positive by inoculation of guinea pigs and two were positive by culture. One culture of acid-fast bacilli, appearing at the end of forty days, appeared identical with cultures of bacilli of tuberculosis, as did one culture of material obtained from a shoulder joint which appeared positive at the end of twenty-four days. In both instances, the comparable pigs were negative. Unfortunately these cultures were not injected into guinea pigs, for they were considered in every way typical. Clinically, neither patient from whom the material was obtained had tuberculosis; the first clearly had adenocarcinoma of the lungs, which was

TABLE 3

COMPARISON OF RESULTS

(All material microscopically negative)

MATERIAL	SPECIMENS	GUINEA PIGS POSITIVE	CULTURES POSITIVE
Urine	84	9	2
Chest fluid	6	2	*
Joints, tissues, etc	10	3	2*
Total	100	14	4

* One specimen of chest fluid and one specimen of fluid from a joint yielded acid-fast bacilli. Both patients from whom these specimens were derived were nontuberculous, clinically and pathologically, and guinea pigs were negative.

proved by surgical removal of a specimen. The second was a typical case of syringomyelia with a Charcot shoulder joint. It becomes evident from these tables that the scarcity of bacilli of tuberculosis in the material delayed the time of the positive cultures considerably, so that from thirty to sixty days elapsed before the diagnosis was made. In some instances the guinea pigs appeared to be positive before the cultures were positive. Guinea pigs were permitted to live until eight weeks had elapsed in this experiment.

A certain number of failures is encountered, both in inoculation of guinea pigs and in cultures; the failures in cultures are due to over-growth of contaminants. Enough over-growth to prevent

TABLE 4

COMPARISON OF RESULTS OBTAINED FROM MICROSCOPICALLY NEGATIVE MATERIAL

TEST	MATERIAL	DAYS BEFORE GUINEA PIG WAS POSITIVE	DAYS BEFORE CULTURE WAS POSITIVE	TUBES INOCULATED	TUBES POSITIVE ACID-FAST BACILLI	DIAGNOSIS WITH REFERENCE TO TUBERCULOSIS
1	Joint fluid (ankle)	28	30	10	1*	Tuberculosis, astragalo-calcaneal joint (surgical pathology)
2	Urine	53	30	8†	3‡	Bilateral renal tuberculosis (clinical)
3	Pus, hip	48	40	8§	1‡	Tuberculosis, hip (clinical)
4	Urine	19	60	8	1‡	Bilateral renal tuberculosis (clinical)
5	Joint fluid (wrist)	33	Neg.	8†		Tuberculosis, wrist (surgical pathology)
6	Chest fluid	25	Neg.	8†		Pulmonary tuberculosis (clinical)
7	Urine	54	Neg.	8		Left tuberculous epididymis (surgical pathology)
8	Urine	53	Neg.	8†		Bilateral renal tuberculosis (clinical)
9	Urine	36	Neg.	8†		Bilateral renal tuberculosis (clinical)
10	Urine	53	Neg.	8†		Same patient as in Test 2
11	Urine	48	Neg.	8§		Bilateral renal tuberculosis (clinical)
12	Urine	53	Neg.	8§		Same patient as in Test 2
13	Urine	31	Neg.	8†		Same patient as in Test 7
14	Chest fluid	56	Neg.	8		Pulmonary tuberculosis, pleurisy (clinical)
15	Chest fluid	Neg.	40	8†	3¶	Adenocarcinoma, lungs (surgical pathology)
16	Joint fluid (shoulder)	Neg.	24	8†	1*	Syringomyelia, Charcot (shoulder) (clinical)

* 1 tube crystal violet-glycerol-potato.
† Some tubes contaminated.
‡ 3 tubes veal-milk-cream-egg.
§ All tubes contaminated.
¶ 2 tubes veal-milk-cream-egg, 1 tube crystal violet-glycerol-potato.

diagnosis occurred in all tubes in 17 per cent of the cases, whereas no failures occurred with use of guinea pigs because at least one animal lived twenty-one days after inoculation. This, however, does not necessarily mean that when one guinea pig survived longer than twenty-one days, in the other guinea pig, which was a failure, tuberculosis might not have developed had the animal lived long enough. Of the 200 guinea pigs inoculated, thirteen

TABLE 5

SUBCUTANEOUS AND INTRAPERITONEAL INJECTIONS 1000 GUINEA PIGS (500 SPECIMENS)

(Only positive animals compared)

METHOD OF INJECTION	RESULT	NUMBER OF SPECIMENS
Subcutaneous	Positive	31
Intraperitoneal	Positive	
Subcutaneous	Positive	6
Intraperitoneal	Negative	
Subcutaneous	Negative	9
Intraperitoneal	Positive	
Subcutaneous	Positive	6
Intraperitoneal	Failure	
Subcutaneous	Failure	6
Intraperitoneal	Positive	

died previous to three weeks. Our records, derived from the routine of two years of diagnostic work, disclosed 4 per cent of failures among cases in which both guinea pigs died previous to twenty-one days after inoculation.

Table 5 contains the results of inoculating 1,000 guinea pigs with 500 specimens of clinical material. These guinea pigs represented consecutive inoculations. The indication is that although a pair of guinea pigs may be inoculated with equal amounts of the same material, it sometimes happens that one is positive while the other is negative, even though they both lived long enough to have lesions.

We believe that as a method of choice in obscure cases in which tuberculosis is suspected inoculation of guinea pigs is still to be preferred to cultural methods. When material is abundant, both methods can be used, providing considerable caution is exercised in the interpretation of the cultures if they are positive before the lesions in the guinea pigs develop or if the cultures are positive and the animals are negative.

COMMENT

The question as to whether guinea pigs, or cultures, or both ought to be used in the diagnosis of obscure lesions of tuberculosis is one that perhaps cannot be settled without taking into consideration the nature of the material and the nature of the institution in which the work is being done. When acid-fast bacilli are found by direct smear, it is usually unnecessary to pursue the matter any further. If it is necessary, the question of the type of tuberculosis present is usually the one involved. Although cultures might sometimes allow distinction to be made between avian and mammalian types of bacilli, the only method so far available for positive distinction of types of bacilli of tuberculosis is by inoculation of animals. If, after acid-fast bacilli have been found, the question arises as to whether they are bacilli of tuberculosis, and the clinical diagnosis is still obscure, one would be forced to inoculation of animals rather than to the use of cultures, since even if cultures were positive, animals would probably have to be inoculated. Hence the decision as to the choice of methods must rest with the results obtained with clinical material which is microscopically negative.

We have demonstrated, as have others, that a fairly considerable number of specimens will produce typical lesions in guinea pigs when the cultures are negative. A few specimens will apparently, in the hands of some workers, produce positive cultures when the guinea pigs are negative, but from our experience it would be quite unsafe to consider such acid-fast organisms as *Mycobacterium tuberculosis* unless they were inoculated into test animals or unless the clinical evidence was positively in favor of tuberculosis. It is admitted that if avian tuberculosis exists in

clinical material derived from human beings, inoculation of guinea pigs will be negative when the culture might be positive, but until the existence of avian tuberculosis in man is on a firmer basis than it is at present, the possibility mentioned must remain as a highly improbable source of error in work with guinea pigs.

The question as to the speed of diagnosis is an important one and evidence now at hand would favor the cultural method. The length of time required for bacilli of tuberculosis to appear in cultures varies considerably. Clairmont[3] reported that most of his appeared in from twenty-five to thirty days, using the modification of Hohn, but he recorded two cases in which they did not appear for about two and a half months. Meyer,[30] on the other hand using the same method, claimed it was unnecessary to wait even as long as ten to twenty days, since by making microscopic examination of smears from the surfaces of the slants, bacilli of tuberculosis could be demonstrated much earlier.

Woolsey[49] obtained many growths in less than ten days, and some as early as two days. Our experience would indicate that growth often does not appear in less than two months, and that it is somewhat correlated with the number of bacilli present in the material. More recently we have had an opportunity to use Herrold's medium and have been surprised to see on two occasions a fairly luxuriant growth of bacilli of tuberculosis, proved to be virulent by inoculation of guinea pigs after four and seven days, respectively. But these were both instances in which large numbers of bacilli of tuberculosis had been demonstrated in the material by direct smear. Although our experience with Herrold's medium is not very extensive, so far as present evidence is concerned one may expect this to yield growth of bacilli of tuberculosis in the shortest time of any medium yet proposed.

It is possible to diagnose tuberculous lesions in guinea pigs in as short a time as fourteen days after inoculation, and almost all guinea pigs that become positive at all will have lesions at the end of thirty days. If guinea pigs are as carefully observed as cultures have to be observed, and if tuberculin testing is employed by experienced persons, diagnosis can be made almost as quickly by guinea pig, and sometimes more quickly than by cultural

methods, when material is microscopically negative. If one demands that the culture be verified by inoculation of animals, the guinea pig method will of course be quicker. Recently, we have been testing guinea pigs as a routine, with 0.5 cc. of "O. T." tuberculin injected subcutaneously at the end of four weeks. So far this has resulted in no deaths of negative guinea pigs and in the death within twenty-four hours of nearly all positive animals. This method has been used extensively at Mt. Sinai Hospital, New York, with excellent results.

In large institutions, where a large proportion of the work is referred to the institution, it is essential that the methods used be as nearly final as possible. This is so important in such institutions that any method for the diagnosis of obscure suspected tuberculous lesions, short of experimentation with animals, is not justified in the light of our present knowledge. Such institutions will not be embarrassed by the added expense of handling guinea pigs, for all of these institutions have to use guinea pigs for other purposes. As a matter of fact, the work of one of us (W. H. F.) indicates that at least two cultural methods should be used on all specimens, if cultures are to be attempted, and he and other workers have brought out the importance of inoculating a large number of tubes. Thus, it becomes evident that the cultural method is not a cheap one, and although it is cheaper than the use of guinea pigs, its cost is not negligible.

If Woolsey and Petrik[50] are correct, twenty tubes must be inoculated in order to equal the dependability of inoculation of guinea pigs. It becomes evident that even to provide incubator room for a large number of specimens will become a problem, and the care in preparing medium and examining it will amount to a sizable item.

In small institutions, where animals are difficult or impossible to keep, cultural methods will find their greatest usefulness. It is perfectly evident that in cases in which clinical evidence strongly indicates a tuberculous condition, demonstration of acid-fast bacilli, either in direct smear or culture, is sufficient confirmatory evidence.

The question of using both methods will find a considerable

amount of support in the evidence presented. However, there is one serious danger, and that is when small amounts of material are available dividing it between guinea pigs and cultures may result in failure by both methods, because of too high dilution of the material.

The medicolegal aspect of the problem is not without interest and importance. At the present time it would be much easier to convince a jury that a given lesion was tuberculous if evidence could be submitted to the effect that in guinea pigs that had been given injections of material from the lesion, tuberculosis developed, than to submit the fact that acid-fast bacilli had been cultured and had not been further tested. The importance of animal controls in such legal questions has been strikingly brought out by the decision rendered by the court in the recent Luebeck disaster, in which the judge held that the lack of controlling the material by inoculation of animals constituted criminal negligence on the part of the chief health officer.

CONCLUSIONS

1. Guinea pigs kept in cages in a room free from tuberculous animals and cared for by healthy caretakers have practically no chance of becoming infected with tuberculosis.

2. Only under the worst conditions will spontaneous tuberculosis develop in guinea pigs, and rarely in less than ninety days. The type of lesion developing under these conditions is characteristic and different from those of experimentally inoculated animals.

3. Spontaneous tuberculosis in guinea pigs that are to be used for experimental purposes can be completely ruled out by exercising reasonable care.

4. Several recently devised methods of culturing *Mycobacterium tuberculosis* are excellent, and can be expected to yield a high percentage of growth in material in which the organism can be demonstrated in direct smear.

5. When bacilli of tuberculosis cannot be demonstrated in direct smear in clinical material, fewer growths will be obtained on culture than can be obtained by inoculation of guinea pigs.

6. Although most acid-fast bacilli that grow on these special mediums will without doubt be virulent bacilli of tuberculosis, the only way positively to prove virulence and identify species, is by inoculation of animals. For this reason, positive cultures are no more final than findings of acid-fast bacilli by direct smear.

7. While cultural methods are valuable and should be frequently used for demonstrating bacilli of tuberculosis, inoculation of guinea pigs remains the best method for proving the presence of virulent *Mycobacterium tuberculosis* in clinical material that is either known or not known to contain acid-fast bacilli.

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DYSPLASTIC GRANULOCYTEMIA*

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Although conditions causing marked leukopenia with concomitant disappearance of the granular leukocytes from the circulation had been described by Brown,[1] Schwartz,[7] and Turk,[8] it was not until the publication of the observations of Schultz[5] many years later that the attention of the profession was focussed on this singular syndrome. Schultz recorded a fatal condition which occurred in females between the ages of thirty-eight to sixty, characterized by a necrotizing mucous membrane involvement of the oro-pharynx, by high fever, slight jaundice, rapid exhaustion, and a very marked leukopenia with concomitant agranulocytosis. His patients had no involvement of either erythropoietic or megakaryocytic systems which explained the absence of anemia and hemorrhagic diathesis. As the number of case reports in the literature rose it became evident that this interesting affection was not a narrow and sharply defined one. Though the basic pathological processes might be the same, the disease manifestations were often found to be different. Thus, cases were described which, though belonging to this symptom complex, instead of oral lesions presented rectal or genital necrosis. Though Schultz originally stated that these cases were rapidly fatal, reports of cures by X-ray therapy, transfusions, and recoveries even without treatment were observed. Although the hematological findings in the early cases showed a complete replacement of the granulocytes by normal lymphocytes, recent publications refer to the presence of monocytes, myeloblasts and pathological lymphocytes.

Many names have been offered to cover the various differences

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in the symptomatic picture or in the physical findings in this syndrome, such as agranulocytic angina (Friedemann) agranulocytic infection (Rose-Houser), stomatitis gangrenosa myelophthisica (Jagic-Spengler), and many others. Schultz[6] believed the term agranulocytosis most applicable because it actually describes the fundamental point in all cases, whereas all other terms describe incidental findings in single cases. The condition described in this paper may belong somewhere in this interesting disease complex and seems sufficiently interesting to warrant publication because it presents some new features of this interesting malady and because a careful study of our data has led us to believe that there must be a definite relationship between this picture and the agranulocytic syndrome.

CASE REPORT

M. K., aged 41, a tucker by occupation, was admitted to the medical service of Dr. A. A. Epstein on October 19, 1930, with the diagnosis of agranulocytosis. He complained of weakness, fever and rectal pain. The patient's friends had noticed his extreme pallor two months before hospitalization. About two weeks before admission he felt that he had fever and also experienced a heavy burning sensation in the region of the anus. Sitz baths, suppositories and other medications applied directly yielded no relief. The patient stayed at his work until two days before admission.

Physical examination. The patient was a well developed, well nourished adult male who though extremely pale, seemed comfortable and not very ill. Nose, mouth and throat were negative. Heart sounds were of good quality, regular, with a slight systolic murmur at the apex which was not transmitted. Lungs were negative. Abdominal examination revealed the liver one finger's breadth below the costal margin. No other viscera or masses were palpable. No rigidity nor tenderness was elicited. The extremities and reflexes were normal. The skin was very pale, soft, of normal texture and showed no hemorrhages.

Just to the right of the anal sphincter there was a black area of gangrene 15 mm. in diameter. Surrounding the lesion was an area of induration. Invasion of the mucous membrane of the anus had just begun.

Progress. During the patients thirty-two day stay in the hospital his sensorium was clear until the day before death. At no time did he develop any physical signs or symptoms other than fever, perianal gangrene, asthenia and pallor which were present on admission. His temperature which was 102.4°F., rose and stayed between 103 and 104 for a period of two weeks. During the last ten days of his life, his temperature was between 99 and 100. The

gangrenous condition gradually increased in size until it reached an area 12 cm. in diameter (fig. 1). This condition eventually destroyed the anal sphincter.

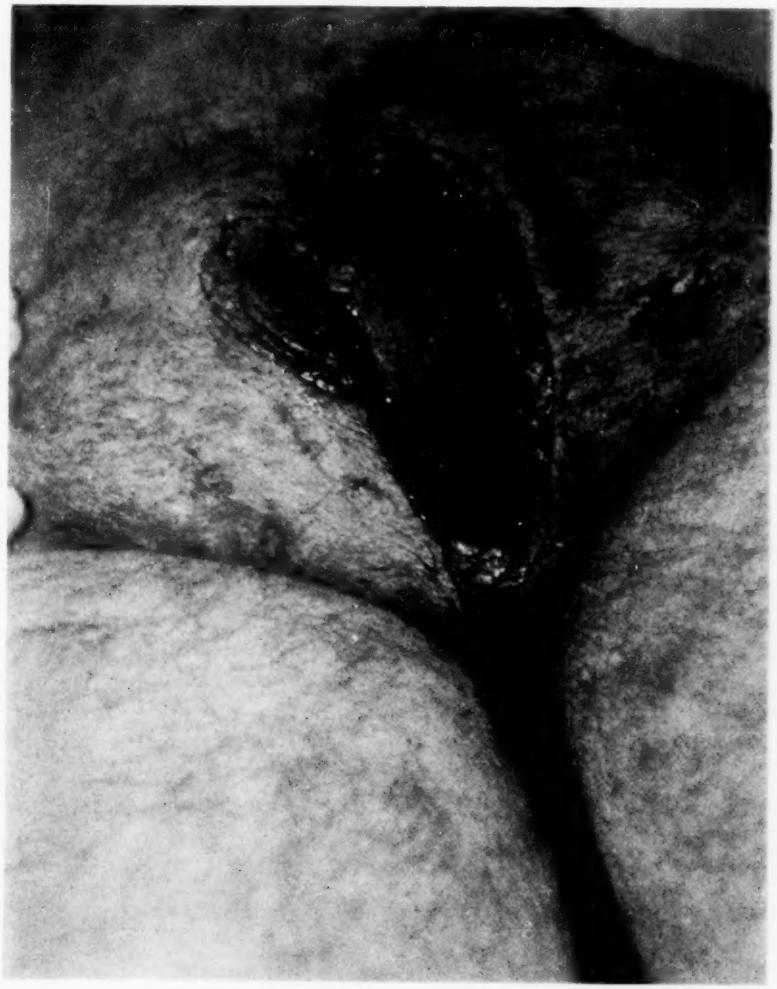

FIG. 1. GANGRENOUS ANAL LESION AS IT APPEARED SHORTLY BEFORE THE EXITUS OF THE PATIENT

Laboratory data. Daily blood examinations were made (see Chart 1). Repeated urinalyses showed nothing of importance. The Wassermann reaction was negative. Many blood cultures were sterile. Blood chemical tests

were normal and the Widal and complement fixation tests were negative. Cultures and smears from the local condition were unimportant.

Inspection of the patient suggested the presence of an abnormal hematological condition. With the knowledge that the patient had a very severe anemia, marked leukopenia and granulopenia the diagnosis became complicated. The

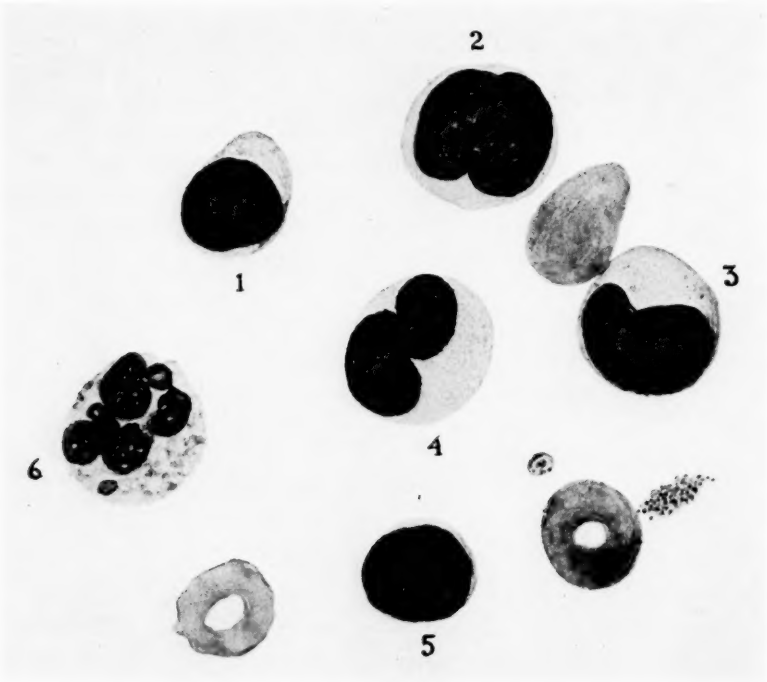

FIG. 2. COMPOSITE PICTURE OF MONOCYTIC CELLS FOUND IN THE CIRCULATION

1, 2, 3 and 4 are different forms of monocytic elements. 5 is a lymphocyte and 6 a neutrophil found before the death of the patient. Cells 1, 2, 3 and 4 show either a lack of or very fine dust-like, granules. The protoplasm varied from basophil to oxyphil in reaction. The nuclei were homogenous showing reticulation. The neutrophil was markedly degenerated.

lack of adenopathy, a splenic tumor, or hemorrhagic diathesis spoke against the ordinary leukemia. The diagnosis of agranulocytosis then seemed plausible.

Careful morphological blood studies however showed many abnormal leukocytes with almost complete absence of the neutrophils. The interesting but confusing element was the presence of 30–40 per cent of mononuclear, apparently

non-granular, leukocytes (fig. 2). The protoplasm of these cells varied from faintly basophilic to oxyphilic, whereas the nuclei were either homogeneous in character, or showed a somewhat granular network. The shape of the nuclei varied from round, oval to kidney and sausage shape. The oxydase reaction was negative. Other of these cells contained very fine, dust-like granules and occasionally some course azurophilic granules. Whereas the basophilic cells simulated atypical myeloblasts, the granular cells appeared to be monocytes. The condition of leukopenia and neutropenia existed for a period of eight days. Thereafter the leukocyte count gradually rose. Bone marrow puncture done on November 3rd showed an almost complete absence of polymorphonuclear elements (fig. 3). A very marked decrease in erythroblastic components was also evident. The predominating cell was a mononuclear cell similar to the monocytic cells encountered in the blood films. Oxydase test was slightly positive. On November 10th neutral red-janus green supravital preparations showed; 0.5 per cent myeloblasts, 2.75 per cent myelocytes—A, 9.25 per cent myelocytes—B, 0.75 per cent myelocytes—C, 1.5 per cent active polymorphonuclears, 76.5 per cent atypical polymorphonuclears; 7.75 per cent lymphocytes and 0.5 per cent monocytes. Neutrophils were atypical in as much as they showed fewer granules than usual and contained a markedly increased number of mitochondria. Their nuclei were also markedly irregular. Progress of the daily blood examinations showed that the monocytic cells seen during the early part of the disease were pathological myelocytes.

Chart 1 illustrates the daily variation in the total number of granulocytes, the percentage of neutrophils and leukocytes. On the date the chart began (October 20, 1930) blood examinations showed 2,000,000 erythrocytes, 40 per cent hemoglobin, 40 per cent pathological myelocytes, 2 per cent "staff" forms, 4 per cent segmented polymorphonuclears, 2 per cent eosinophiles, 43 per cent lymphocytes and 9 per cent monocytes. As already noted this picture remained essentially the same, with some fluctuations until October 28, 1930 when the number of granulocytes began to increase, the pathological myelocytes to diminish and the segmented polymorphonuclears to increase. There also began a dimunition of the lymphocytes. Examinations on October 29 revealed 2,140,000 erythrocytes, 40 per cent hemoglobin, 27 per cent pathological myelocytes, 7 per cent "staff" forms, 34 per cent segmented polymorphonuclears, 22 per cent lymphocytes and 10 per cent monocytes. This tendency continued until at the time of death the patient had 1,860,000 erythrocytes, 34 per cent of hemoglobin, 2 per cent pathological myelocytes, 10 per cent "staff" forms, 90 per cent segmented polymorphonuclears, 6 per cent lymphocytes and 2 per cent monocytes. It is of interest to note that during the patient's last week of life his leukocytes rose to 40,400 and the neutrophils to 92 per cent. These granulocytes were markedly vacuolated, the nuclei very irregular, and their granules disordered and poorly staining.

Treatment. During the patient's stay in the hospital he received Sitz baths and various antiseptic dressings for his local condition. At no time did this

condition show any improvement. For his anemia he received three transfusions of 500 cc. each, liver extract, Blaud's pills and spleen marrow. He was

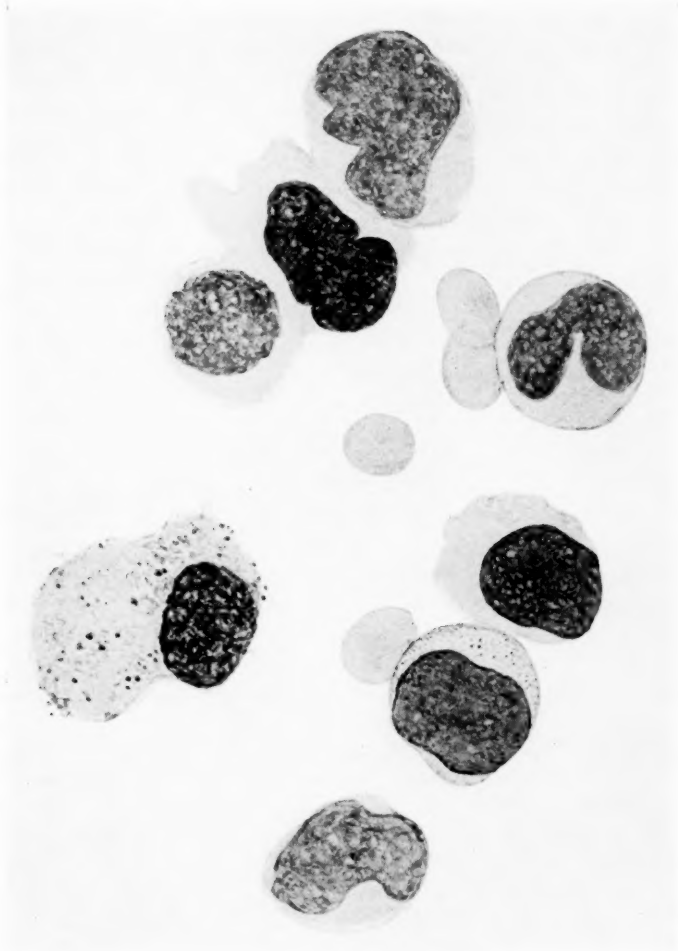

FIG. 3. CELLS FROM THE BONE MARROW

Mononuclear finely and irregular granular leukocytes. Erythroblastic elements markedly diminished. There was an absence of polymorphonuclear neutrophils.

also given five injections of neosalvarsan. In spite of all therapeutic measures the patient, complaining of nothing except weakness and slight anal pain, grad-

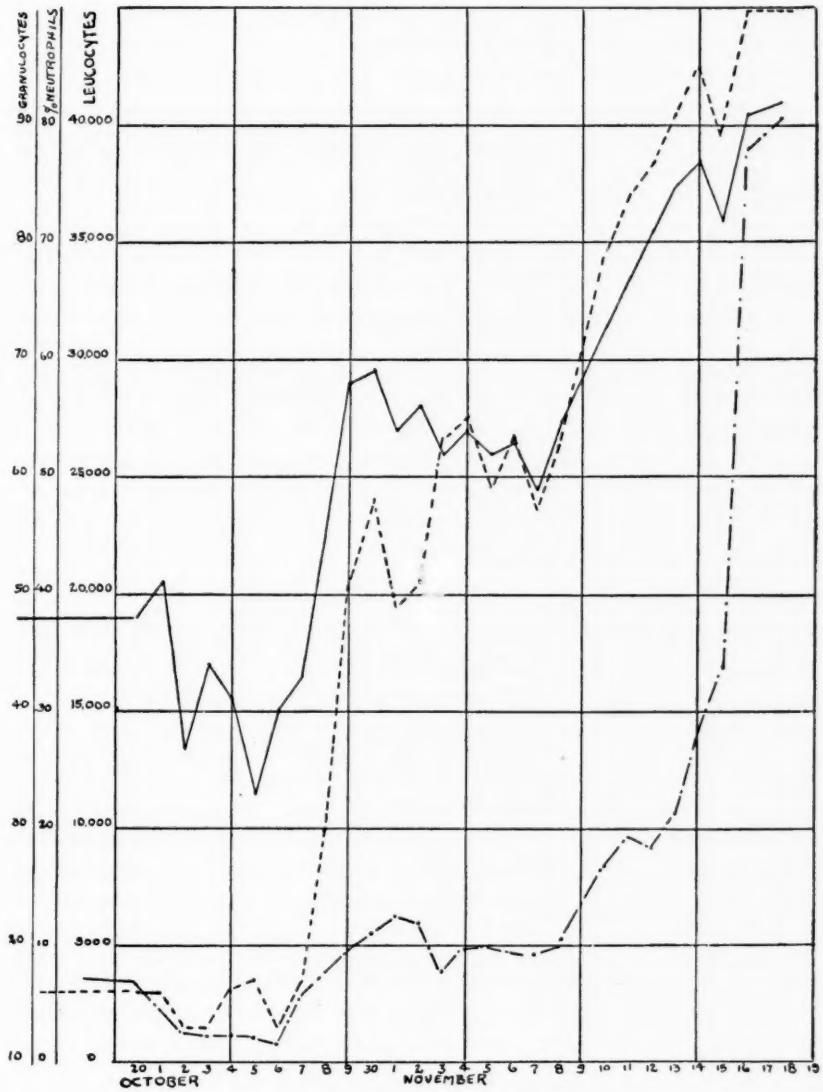

CHART 1

ually grew more asthenic, and died. At the time of his demise his temperature was normal and the neutrophils had returned in large numbers in the circulation. During his illness he had shown no sign of a suppurative process, nor had he developed a hemorrhagic diathesis. A bone marrow puncture wound which had

healed by first intention, broke down one week before death. Smears taken therefrom showed numerous cocci in the reddish gray non-purulent discharge.

Autopsy findings. The body was that of a very pale tall strongly built man with slight edema of the lower extremities and a large black necrotic mass encircling the anus. This mass covered an area 7.5 cm. wide by 12.5 cm. long and extended deep into the tissues. There is no swelling of the lymph nodes. Heart: post mortem coagula were of a peculiar reddish color. Closing margins of the mitral valve show fine veruccae. Spleen 225 grams 16 cm. × 10 cm. × 3 cm. shows a typical wedge-shaped anemic infarct 1.3 cm. × 1.3 cm., on its lower margin. Splenic vessels were intact. There was a similar infarct at the other pole. Cut surface showed nothing unusual. The left kidney had many small hemorrhages which coalesced to form patches. Cut surface indistinct. Near the upper pole there was a mixed infarct 1 cm. deep. There was a thrombus occupying a large vein. The right kidney showed a small anemic infarct near the lower pole with a larger one near the upper, no thrombi. There were many submucous hemorrhages in the lower third of the ureter. The gasto-intestinal tract was normal except for 4 cm. of rectum near the anus which was blackish green and foul smelling. Rib bone marrow normal in porosity, extremely pale; vertebra similar, tibia (at one point) was fatty gelatinous, with very little marrow.

Microscopically the kidney showed no fatty changes, a large venous branch was thrombosed. The thrombus contained disintegrated red blood cells and many leukocytes, which were not normal. They were irregularly shaped. Surrounding the thrombus there was a wide and irregular infiltration of mononuclear elements. Many were typical plasma cells simulating a picture of acute interstitial nephritis. The glomeruli contained very little blood. The kidney showed very few hyalinized glomeruli and very little inflammatory infiltration. The splenic infarct gave the identical picture. There was nothing unusual in the cellular content of the spleen, and lymph nodes. The bone marrow of the tibia was mostly fatty, contained many indefinite mononuclear elements with dark lobulated nuclei, no erythropoiesis. A vertebra was cellular thruout, few blood vessels found, but many megakaryocytes and large mononuclear cells similar to those of the blood film were identified. The nuclei of these cells varied from round, ovoid to crescent and kidney shaped, bilobed or with many lobes. Whereas no normal neutrophils could be seen in the bone marrow, small groups are occasionally seen in the lumen of the vessels. There were few erythroblastic components.

DISCUSSION

How does the condition described compare with the agranulocytic syndrome? A review of the literature reveals as many observers who believe that agranulocytosis is primary and sepsis secondary, as those who think the sepsis is primary. Exclusive of these are the cases of paralysis of the granulopoietic apparatus

following arsenic therapy, excessive X-ray therapy and after benzol. Cases of bone marrow exhaustion have also been encountered in individuals with debility. Our patient was a strong well developed man who had had no illness. Even careful post-mortem examination revealed no possible etiological cause for this condition. Except for the extensive anal necrosis the intestinal tract was normal. The findings described by Koch,[4] of a large series of post-mortem studies showed thrush-like lesions of the digestive tract with oropharyngeal necroses. It has been suggested that some agent such as faulty food may exert some selective action on the intestinal tract and secondarily on the bone marrow. The lesions in our case were in no way comparable with the findings of Koch. We feel that this case speaks for a primary affection, with an unknown causative agent.

Just what initiates the symptomatology of this condition? Bone marrow examinations in the majority of cases reported seem to indicate, in spite of their lack of uniformity, a disturbance of the bone marrow which results in a curtailment of the production of granulocytes. David[2] believes that the disturbance is not of production, but of outflow distribution. In our case we are not dealing with an agranulocytosis but with a condition which the granulocytes formed are imperfect in structure. As an inspection of the table will show, there were 33–92 per cent granulocytes present at all times, even when the leukocytes had dropped to 700 and the neutrophils to 3 per cent. The neutral red-janus green stains definitely placed the pathological mononuclears as myelocytes with deficient granules. The subsequent course of the blood count showed that these cells were the precursors of poorly granular and vacuolated neutrophils. Thus it appears that the condition resulted in the production and distribution of imperfectly constructed neutrophils which we believe were unable to assume their normal functions. For this reason we have selected the term dysplastic granulocytemia.

In the rapidly fatal cases reported in literature the granulocytes are either markedly decreased or totally absent, with a complete replacement by lymphocytes. In these cases the bone marrow is paralyzed and the granulopoietic function ceases. In

other cases, including the one under discussion, the bone marrow injury though not paralyzing, is irreparable and results in the disordered formation of granular cells. Ordinarily if the duration of the illness is protracted there is the return of what appear to be normal neutrophils. Studies of the neutrophils that appeared during the last week of our patient's life showed marked vacuolization of the protoplasm with toxic degeneration of the nucleus and granules. Whereas a marked leukopenia persisted during the early part of the illness, during the latter part, the leukocytes rose steadily. Coexisting with this severe leukocytic picture was a profound anemia. With the rise of the leukocytes there appeared a corresponding rise in the neutrophils.

Those who have reported recoveries in agranulocytosis have stressed the return of the neutrophils as the cause of recovery. Koch found the return of neutrophils with resultant recovery in 15–25 per cent of all his cases. Lack of neutrophils is considered incompatible with life. Thus X-ray therapy, transfusions, vaccines, et cetera have been used with apparent success to cause by stimulation of the bone marrow a return of the neutrophils to the circulation. Friedeman and Elkeles believe that the efficiency of X-ray therapy in cured cases can be seen by the increase in bone marrow elements during the first twenty-four hours after treatment. In our case however the return of an increased number of neutrophils did not alter the subsequent course. Every hematologist has noticed the presence of degenerated neutrophils similar to those observed in our case, somewhat before and after the peak of an acute infection. With the infectious process overcome these degenerated cells gradually disappear and the bone marrow provides normally constructed and functioning cells. During the period of stress cells as young as the myelocyte are often set out. In our case the bone marrow relieved of its pernicious influence, was able to produce neutrophils in huge quantities, but of deficient quality, because of an irreparable injury to the mother cell.

With the employment of the ordinary staining methods we believe that it is not at all unusual that these cells may be either overlooked or improperly interpreted. In this connection the

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TABLE 1

SCHEMATIC REPRESENTATION OF THE ESSENTIAL DIFFERENCES FOUND IN SOME OF THE HEMATOLOGICAL CONDITIONS

DISEASE	ERYTHROCYTES	LEUKOCYTES	THROMBOCYTES	HEMORRHAGIC DIATHESIS	PROGNOSIS
Agranulocytosis	Usually normal; below 4 million in 39 per cent of cases	Disappearance of neutrophils; often completely absent	Usually normal	Usually none; present in 17 per cent of cases	Death unless granulocytes return in normal numbers
Aleukia	Decreased	Decreased	Decreased	Present	Death
Purpura hemorrhagica	Severity of anemia depends on the amount of hemorrhage	Increased	Decreased; may be totally absent	Present	Condition controlled by splenectomy
Pernicious anemia (Addison-Biermer)	Decreased	Decreased; relative lymphocytosis	Usually normal; decreased in terminal states	Usually none; except in terminal stages	Good with adequate therapy
Secondary anemia	Decreased	Increased	Increased	None	Good with removal of etiological cause
Dysplastic granulocytemia	Decreased	Disturbed formation and function of the granulocytes. Varies from severe leucopenia to marked leucocytosis	Normal	None	Death in spite of return of granulocytes

supravital stains are of utmost importance. Table 1 summarizes the important differential points in separating several hematological conditions.

SUMMARY AND CONCLUSIONS

We have described in detail a case which demonstrates a symtom complex somewhat similar to that already described by Schultz and others as agranulocytosis. Detailed scrutiny of our case has revealed definite basic differences. The term dysplastic granulocytemia has been suggested to designate this idiopathic affliction occuring in an apparently healthy individual. It is characterized by a primary involvement of the granulopoietic system with the resultant production and distribution of imperfectly constructed neutrophils. Whereas the disappearance of the granulocytes is the central concept of agranulocytosis we encountered different findings here. Although the polymorphonuclears had dropped to 3 per cent and the leukocytes to 700, we found that during the course of the illness, granulocytes were always present amounting to between 32 and 93 per cent of the cells. Such being the case the term agranulocytosis cannot fit. Notwithstanding the fact that the leukocytes rose to 40,400 and the neutrophils to 93 per cent, and no intercurrent infection apparently intervened, the patient died. Instead of the small anal gangrenous condition improving with the return of the neutrophils, it gradually grew larger. Heretofore all of the cures in cases of agranulocytosis were attributed to the return of the granulocytes, whereas in this case a marked leukocytosis did not prevent death. The death in this case can only be explained by the inability of the neutrophils, although present in larger numbers, to assume their normal function because of an inherent deficiency in their formation.

It is because of these differences that we believe that the term dysplastic granulocytemia is more suitable for that group of cases that may fall within the description we have given. We feel that a careful morphological examination of future cases will tend to show that many of the patients presumably suffering with the agranulocytic syndrome, are in reality examples of dysplastic granulocytemia.

We wish to thank Dr. Florence R. Sabin, of The Rockefeller Institute, for her aid with the supra-vital stains, and Dr. I. W. Held for his kind suggestions.

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CLASSIFICATION OF LEUKEMIAS*

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Since routine blood counts have been introduced in hospital practice the number of recorded leukemias is rapidly increasing. It is evident that this growth is not due to a greater incidence of the disease, but to the more accurate methods of diagnosis. Leukemia can no longer be classed as a rare disease, at least by the laboratory worker.

When a diagnosis of leukemia is established, it is important to determine whether it is of the acute or chronic variety. The clinical course differs greatly in the two types. The immediate prognosis of the chronic type is generally good; the duration may be five to eight years. On the other hand, the prognosis of an acute leukemia is very grave. Seldom is the course longer than five to six months. In the majority of instances, the patient succumbs in one or two months.

The typing of leukemias into lymphoid and myeloid is of some value in the chronic leukemias. If radiation be employed as a method of treatment, the radiologist may be guided by the type of leukemia in order to concentrate on some particular tissue. This is the strongest argument in favor of the genetic typing of chronic leukemias. Aside from that, the value of the typing is mainly corroborative; if the disease is associated with splenomegaly, one would expect to find the myeloid type and if the glandular enlargement is conspicuous, the lymphoid variety is the usual finding. There are exceptions to this rule, though uncommon. The differentiation of the cell types in acute leukemia (if it could be successfully done) has no prognostic or other clinical value.

The clinician should accept the diagnosis "acute leukemia" as

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a final and complete entity. Therapeutic measures would be the same regardless of the histogenetic type.

The clinical course is always the same and the logic of this subdivision even from a histogenetic viewpoint can be questioned. Any attempt to classify acute leukemias on the basis of cell types usually involves a good deal of argument, and the explanations are not always entirely acceptable.

Similar views were expressed by me[12] in a previous paper. Not possessing the authority of an academic histopathologist I could but mildly stress such views. The previous paper was based on the clinical observations and the hemotologic studies of five cases of acute leukemia. Six more cases of acute leukemia and a number of chronic cases have been studied since then. Every new case observed corroborated the above contentions, as well as do many statements in literature.

The typing of leukemias into lymphoid and myeloid is interwoven with the different theories of the origin of the blood cells. The histologist frequently seeks in leukemic cells explanations or proofs for his theories about the hematopoietic tissues. The pathologist must study the literature of the histologist and embryologist when analyzing leukemias.

A. Fränkel[4] (cited by Hirschfeld)[6] was the first to describe and formulate theories regarding acute leukemias. Naturally, he expressed only the knowledge of his times (most of his articles appeared between 1895–1898). Fränkel emphatically stated that the diagnosis of "acute leukemia" can be made from the blood picture alone. The predominance of large lymphocytes plus a leukemic high total leukocyte count was sufficient evidence to make such a diagnosis. Furthermore, Fränkel and many other contemporary hematologists considered the large lymphocyte as the mother-cell of all the leukocytes of the blood. Fränkel's views were directly opposed to Ehrlich's. The latter, a few years after Fränkel's work, laid down his well-known views. Ehrlich, Lazarus and Pinkus divided the white cells of the blood into the lymphocytes on one side and the granulocytes on the other. According to these authors lymphatic leukemia (acute or chronic) was a disease of the lymphoid tissue only. They were especially

opposed to the view that the bone marrow is also involved in lymphatic leukemia. Newman, whose views were later accepted by Walz and Pappenheim, considered the bone marrow involvement a necessary accompaniment for any leukemic blood condition. Hirschfeld[5] cited about a dozen authors, including Pappenheim, who reported cases of leukemia in which the bone marrow alone was involved. The term "mixed leukemia" was introduced. This term expressed the opinion that both myeloid and lymphoid tissues were involved. This term is often used in the German literature synonymously with "myeloid leukemia."

Summarizing his article on acute leukemias, Hirschfeld[6] expressed the opinion that taking in consideration the frequency of transition from one type of leukemia to another, one must deduct that all the leukocytes have a common origin. In an article, published a year later, he[7] discussed the histopathology of leukemia from the standpoint of both unitarian and dualistic theories.

The following passages from that publication are noteworthy:

> Our views about the morphologic classification of leukemias depend on the basic views about the histogenesis of leukocytes. As is known, Ehrlich, and with him many authors, accept a strictly dualistic view point. The possibility of lymphocytes being generated by the bone marrow is not admitted by them. While Ehrlich admits that there are granule-free mononuclear cells in the bone marrow, he absolutely denies that the spleen and lymph glands can generate granulocytes. He (Ehrlich), therefore, considers lymphatic leukemia as an involvement of the spleen and lymph glands only and the mixed-cell (myeloid) leukemias as an involvement of the myeloid tissue in the bone marrow. The undeniable fact that lymphocytes may be found in the bone marrow in lymphatic leukemia and that myeloid elements are found in the spleen and lymph nodes in myeloid leukemia is explained by Ehrlich on the basis of metastases that is, the blood stream will transport and colonize myeloid elements in lymphoid tissue in cases of myeloid leukemia and vice versa. On the basis of my observations [concluded Hirschfeld], I formulated different opinions which should be called unitarian.

The introduction of the myeloblast by Naegeli (a non-granular cell of the bone marrow) as the parent cell of the granulocytes was supposed to refute the claims of some contemporary hematologist (especially Pappenheim) that the "lymphoid cells" of the marrow are identical with the lymphocytes of the blood and lymphoid

tissue. Supposedly a different cell, the lymphoblast, was discovered to be the parent-cell of the lymphoid elements. This cell, morphologically very similar to the myeloblast, was claimed by Schridde to possess certain biologic characteristics distinguishing it from the latter; and so the myeloid and lymphoid elements were completely divorced. The position of the dualists seemed to be unvulnerable.

The similarity of the lymphoblast and myeloblast, however, is so great, that even in the camp of the dualists soon expressions were made about the difficulty to differentiate them. Butterfield, Heineke and Meyer,[1] (cited by Downey[3]) although accepting the dualistic views, stated that all the morphologic characteristics claimed for the lymphoblast by Naegeli and Schridde were also to be found in the myeloblast, including the Altmann-Schridde granules.

The adherents of the strict unitarian view went one step further. They claimed that the lymphoblast and myeloblast were one and the same cell. Minor biologic characteristics could be explained, they contended, by the fact that they were growing in different tissues. Maximow[9] cultured lymphoid tissue in blood plasma, to which an extract of bone marrow was added. The large lymphocytes were seen to divide by karyokinesis and to differentiate step by step into (1) leukoblasts or promyelocytes, (2) granule-poor myelocytes, (3) typical granule-rich myelocytes and (4) myelocytes with horseshoe-shaped nuclei.

Maximow considered these findings as experimental proof that myelocytes will originate from lymphoblasts in a myeloid medium. He concluded that the dualistic theory obscures and complicates our conception about haemopoiesis. The unitarian teaching has much simpler explanations. Histologically identical, lymphoblasts and myeloblasts are considered the same,—being one and the same cell.

Between the extreme unitarian theorists and the strict dualists there are many noted histologists who accept a modified view.

Granting that in the healthy adult human and other mammals there exists a morphologic dualism, authors agree that in pathologic conditions, especially in leukemias, metaplasias are very

common. Downey,[3] who can be classed as a moderate dualist, agrees with Naegeli that the myeloblast is a real stem-cell of the meyloid elements of the marrow, while the lymphocytes of the lymph nodes and the spleen are regenerated by mitosis of their own kind without the intervention of the myeloblasts. Yet he takes a strong stand against Naegeli's dogmatic dualism. He attacks Naegeli's points of differentiation between a lymphoblast and a myeloblast on morphologic, biologic and histologic grounds.

It is out of the province of this paper to argue for or against any given theory of haemopoiesis. Enough had been mentioned to justify the conclusion that in leukemias, at least, a sharp line cannot be drawn between the stem-cells of the lymphoid and myeloid tissues.

It is an established fact that in the leukemias myeloid cells may be found in lymphatic tissues and vice versa. The limitations, put by nature upon healthy adult cells to develop their own kind only, do not exist in leukemias. The hemopoietic tissues revert to their embryonal state. According to Lang,[8] myeloid transformation occurs especially freely and frequently in those organs which generated myeloid elements in the embryo, namely the spleen, lymph nodes, liver, thymus, kidney and adrenals. The metaplasia is considered to be a return to the foetal blood-forming function of these organs. Furthermore, the metaplasia is autochtonous, that is it comes from certain cells of that tissue itself, not colonized by the blood stream from some distant focus. The myeloid cells develop from certain lymphoid cells, haemocytoblasts; these under physiological conditions produce only lymphocytes in lymphatic tissue, never myelocytes.

The following statement is frequently found in literature: "Though the case reported had all the characteristics of acute lymphatic leukemia, the post-mortem findings revealed it to be acute myeloid leukemia." The revelation is generally based on the myeloid infiltration of the spleen, lymph-glands, et cetera. If one could prove that the bone marrow was the only tissue involved at the onset and the lymphoid tissue secondarily colonized by the myeloid cells through the blood stream, there would be some basis for such a stand. Such proof is so far lacking.

Acute leukemias show involvement of the bone marrow, spleen, lymph nodes, liver, kidneys and other organs, in varying degrees. The differentiation of cell types is no more distinctive in the tissues than in the blood. The cells are usually large mononuclears; the nucleus comprising about 90 per cent of the entire cell. Only a narrow border of cytoplasm is present with an excentric nucleus. From two to five granules are scattered at the periphery of the nucleus. The density of the nucleus is very uneven. In the healthy adult such a cell is altogether foreign to lymphoid tissue. Similar cells may be found in the bone marrow in greater or lesser numbers. Hence the conclusion that it is a myeloid cell and the leukemia is typed as myeloid. Why not go one step further and conclude that it is an embryonal cell, a blastic cell, which characterizes the leukemia as one of the malignant acute variety? To argue that this blastic cell belongs genetically to the lymphoid or myeloid tissue often leads to a fruitless argument. First we want to establish the criterion which identifies a cell as myeloid or lymphoid.

If the criterion for considering it myeloid is based on the assumption that it was generated by the marrow, the name is fallacious. Enough proof had been cited to show such fallacy. If the criterion for considering it myeloid is based on the morphology of the cell, it has not yet been done by any worker with a reasonable degree of certainty.

As Maximow[10] puts it, "The morphologic variations between two myeloblasts are often greater than that between a myeloblast and lymphoblast." (Cited by Downey.[2]) The biologic differences, especially those based on the oxydase reaction, are uncertain. When Winkler established this reaction as characteristic for myeloid cells only, many authors of the polyphyletic school accepted it as final for differentiation of cells of doubtful morphology. At the present the specificity of this reaction for myeloid cells is denied by many. Menten[11] (cited by Downey[3]) concluded that the reaction was an absorption phenomenon dependent on properties of intracellular surfaces, and that lymphocytes gave also a well marked reaction. Graeff[5] considered this reaction common for all kinds of cells of the animal organism. He con-

cluded that the oxydase content of the cells is more quantitative than qualitative. This fact (according to Graeff) leads some observers to hasty conclusions that this or that cell does not contain any oxydase.

Gunther Wollback[14] differentiated between endogenous and exogenous oxydase. By injecting horseradish extract into the subcutaneous tissues of a white mouse an inflammatory reaction was produced and an oxydase deposit was obtained in the different types of phagocytic cells concerned in the reaction, myeloid as well as non-myeloid (histiocytes and fibrocytes). An extract of bone marrow gave similar results, although not in the same degree. He concluded that no genetic grouping of the cells should be undertaken on the basis of the oxydase content.

From a review of the literature on acute leukemias and from his own observations on twenty-eight new cases Warren[15] drew conclusions that almost coincide with those expressed here. Noteworthy are the following passages:

> There is no essential difference in the clinical picture of acute leukemia, whether the case has been diagnosed as acute myelogenous or lymphogenous leukemia. The wide divergence in diagnosis in all these cases is due to the difficulty in differentiating the immature cell-forms so that it is practically impossible to separate the cases into the usual two types.
>
> Apparently most of the acute cases on record that have been diagnosed as lymphogenous could just as well be called myelogenous leukemia or vice versa, because there is no definite difference between them in the onset, progress, signs and symptoms, blood findings and autopsy descriptions. There are differences in degree only.

Warren, however, cannot discard the traditional conception that with the aid of special stains, in expert hands, specific characteristics of leukemic cells will be demonstrated. He considers the supravital staining method of Sabin and her coworkers as the criterion of differentiation. He refers to a case of myeloblastic leukemia reported by Sabin, Austrian, Cunningham and Doan,[13] in which this method of staining determined the type of the leukemia. This case was first considered to be an acute lymphatic leukemia, on account of the predominance of cells "apparently of the lymphocytic series" in the blood. By this special method of

staining these cells were observed to mature by amytotic cell division and give birth to a series of cells, considered by Sabin to be myelocytes. The cells which these expert histologists considered to be "apparently of the lymphocytic series" proved to be myeloblasts, because oxydase-positive cells were reproduced by them. It seems more logical to me to postpone the final judgment on the primary cells until someone demonstrates a similar case in which the supravital staining will reveal mature lymphocytes to be the progeny instead of the myelocytes. Then only will we have conclusive proof that the blastic cells observed were myeloblasts in Sabin's case and lymphoblasts in the second instance. It may be of interest, that about one year later[2] the same authors in another publication on the development of the different forms of white cells from a common stem-cell, express themselves as follows: "The granulocytic leucocytes, monocytes, and lymphocytes are all formed from a single stem-cell."

The discussion heretofore was centered on the two main genetic types of cells: lymphoid and myeloid. Now a third cell type, the monocyte, an offspring of the reticulo-endothelial cells is distinguished. Cases of monocytic leukemia and reticulo-endothelial leukemia have been reported.

It is also my belief that there is no need for singling out reticulo-endothelial or monocytic leukemia as a separate entity. If the majority of the cells are blastic cells, characterized by large nuclei with a sieve-like reticulum and several nucleoli, the diagnosis of acute leukemia is justifiable. If the cell is more or less round or slightly oval, with a narrow border of cytoplasm to one side of the nucleus, it may be identified by some of the polyphiletists to be a promonocyte and by others a lymphoblast or myeloblast. There really is no morphologic distinction. The unitarian or monophiletists would class it as a haemocytoblast, and do away with all the other three possibilities. Occasionally one sees a leukemia in which the cells have the same type of blastic nucleus but the cytoplasm is very irregular in outline and very voluminous in proportion to the nucleus. The entire cell may reach enormous proportions, the increase being chiefly in the cytoplasm. This originates from the reticulo-endothelial tissue and is known as a

histiocyte or clasmatocyte. It is supposed to be the parent-cell of the monocyte. In one of my last cases there was a predominance of these cells in the blood-smear. The patient was a man in the early sixties. He was admitted to the hospital with a diagnosis of "ulcerative colitis," because of the discomfort in the abdomen and the constant bloody evacuations. The leukocyte count on admission was 16,000. The smear, as mentioned, showed chiefly these peculiar cells and some mature lymphocytes; no granulocytes were seen. I considered the blastic appearance of the nucleus sufficient evidence to warrant a diagnosis of acute leukemia, disregarding the enormous size of the cell and the fantastically shaped cytoplasm. The case proved to be a highly malignant form of acute leukemia. The man lived only a little more than two weeks. Towards the end, his total leukocyte count was 60,000. The cells resembled haemocytoblasts, such as are seen in the more common types of acute leukemia.

Many observers are inclined to classify such a case as a monocytic type. From the standpoint of descriptive exactness it is perhaps in such rare types indicated, advantagenous to mention the unusual morphology of the cell. Although from every other standpoint, one should always remember the main point, namely, if the nucleus of the cell is of the blastic type, it is an acute leukemia. If the nucleus is of the more mature variety it is a chronic case. In the latter case the differentiation of the cell type is comparatively simple, and therefore, the genetic classification is possible.

Let me emphasize the important points in the diagnosis of leukemias as follows: if the majority of the cells are of the immature blastic type, as evidenced by the appearance of the nucleus, the diagnosis of acute leukemia is justified. No other classifications are necessary, irrespective of what beliefs one has about the histogenesis of the blood cells in healthy adult tissues. If, on the other hand, the cells are relatively mature, judged mainly by the absence of the nucleoli in the nucleus, the diagnosis is chronic leukemia. In the latter instance, the cells are generally sufficiently differentiated to enable one to establish the cell type.

The heretofore accepted classification of acute leukemia, ac-

cording to cell types, has no clinical value whatsoever; and even the correctness of the cytological classification in many of the reported cases is questionable. If we desire to be more radical, and to discard altogether the traditional classifications, a more exact and descriptive classification would be: "blastic" for the acute forms and "cytic" for the chronic forms. To avoid the possibility of being involved in any academic disputes, the term haemocytoblast is the most acceptable for the cell which characterizes acute leukemia. And so we could name the disease "haemocytoblastic leukemia." The chronic leukemias would always be denoted by the -cytic ending with the exact cell type modifying the nomenclature, for example, lymphocytic, myelocytic. The endings -genous and -oid, as myelogenous or lymphoid should be avoided, as confusing, if the more descriptive classification into "blastic" and "cytic" is accepted. Occasionally one sees a case of chronic leukemia of either variety, which, after running a mild course for several years, turns into the more malignant or blastic type. The blood picture then is that of a true acute leukemia. The fact that a particular case has been diagnosed in the past as chronic leukemia should not influence the pathologist; he should be guided only by the present findings. The prognosis in such cases is the same as in any other acute leukemia.

SUMMARY AND CONCLUSIONS

(1) The acute leukemic syndrome is always characterized by the same history, clinical course, blood findings and tissue changes. There are differences in degree only.

(2) The typing of acute leukemias, into myeloid and lymphoid does not add anything from a prognostic or any other standpoint. The accuracy of this typing, even from a histogenetic viewpoint, is questionable.

(3) There is no logical reason for the division of acute leukemias into different types, unless for the sake of perpetuating traditions.

(4) When the diagnosis of "acute leukemia" is made, it should sound sufficiently expressive to the clinician, from a diagnostic,

prognostic and descriptive standpoints. If a more descriptive expression is desired, the term haemocytoblastic leukemia may be recommended, indicating that blastic cells, stem-cells, are dominating the picture.

(5) For the chronic leukemias the old genetic typing may be retained because the cells are relatively mature and more differentiated.

(6) If the term haemocytoblastic be accepted for the acute leukemias, the genetic typing of the chronic variety with -cytic ending would be proper, such as lymphocytic or myelocytic.

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DISCUSSION

DR. MAX M. STRUMIA, Philadelphia, Pa.: Doctor Rubnitz' paper has indeed a number of excellent points on which I thoroughly agree with the author.

In a paper read in the fall of 1930 before the Philadelphia County Medical Society, I expressed exactly the same view in regard to the unimportance of the differentiation between acute lymphatic leukemia and acute myelogenous leukemia. I believe that there is but one form of acute leukemia and that the differentiation between the two, rather than being useless, as Dr. Rubnitz expressed himself, is erroneous.

I cannot agree with Dr. Rubnitz, however, in his remarks about the oxidase reaction. The oxidase reaction is ordinarily done without proper controls and being a rather empirical test, controls of normal fresh blood should always be carried alongside the patient's blood. I have repeatedly found, for instance, that if one follows the course of a case of acute leukemia, cells that are oxidase positive in the early course of the disease will not show the oxidase granules later. The oxidase reaction, is therefore, not to be discarded but rather to be done more carefully and its results more critically interpreted.

Nor do I agree with the common conception that acute leukemia is simply a phase of chronic leukemia or a rapidly going form. The two diseases differ vastly; the chronic leukemia being, undoubtedly, a neoplastic disease, characterized by the enormous increase of immature but differentiated cells of either the lymphatic or myelogenous series. Acute leukemia, is essentially a very acute breaking down of the bone marrow (erythrocytes, granulocytes and platelets alike being affected) with a secondary, variable, compensatory hyperplasia of either lymphocytic or monocytic (reticulo-endothelial) systems. In addition, the abnormal cells of acute leukemia are *always* indifferentiated cells.

While I agree that the name, acute leukemia, is very undesirable, yet I am personally opposed to the introduction of new terms; it is sufficient to mention that for the primordial or indifferentiated cells found in the circulating blood of patients with acute leukemia at least ten different names have been used.

THE SEPARATION FROM BLOOD GLUCOSE OF TWO NON-GLUCOSE REDUCING SUBSTANCES*

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The quantity of sugar apparent in the blood as determined by such methods as the Folin-Wu and the Myers-Bailey, includes besides the true glucose, a non-glucose reducing substance. The numerous methods for blood sugar estimation brought out in the last fifteen years have been designed with the object not only of eliminating the reduction caused by creatinin, uric acid, et cetera, but of making a close approximation to the true glucos content of the blood. Glucose may be removed from the blood in a few seconds by years fermentation and measured by the loss in reduction.[6, 7, 18] The true glucose may also be measured directly by the new Myers-Root[11] method in which the interfering substance is precipitated by zinc, or it may be determined by the Folin-Wu reagents after precipitation of this substance with the blood proteins by the copper precipitation** of Somogyi.[17] The standard methods for blood sugar are correctly accepted as clinically accurate because this non-glucose reduction is practically constant in amount in health and disease.[19] The normal range of blood sugar is about 30 mgm. per 100 cc., the normal amount by the Folin-Wu and Myers-Bailey methods is 90 to 120 mgm. per 100 cc., by the Benedict copper method 75 to 100 mgm.

* Read before the Section on Pathology, California State Medical Association, San Francisco, 1931.

** One volume of blood, 7 volumes distilled water, 1 volume 7 per cent copper sulphate solution. Shake. Add 1 volume 10 per cent sodium tungstate drop by drop, shaking with each addition. Filter. Determine the glucose in the filtrate by the Folin-Wu method.

GLUCID X

The amount of true glucose in blood is the same as determined by all methods, but the non-glucose substance, estimated as glucose, varies in amount by the different methods of oxidation. Fontès and Thivolle call this non-glucose reducing substance glucid X; it is the non-glucose substance described by Best[1] as containing a pentose fraction, as not being fermentable by yeast, and as being in part precipitated by phosphotungstic acid. Glucid X does not react to the reagents of Fontès and Thivolle after fermentation, and they considered it destroyed with the glucose measuring it by the difference in reduction in the tungstate and zinc filtrates. Measured by the Folin-Wu method as the difference in reduction before and after yeast fermentation* glucid X averages about 20 mgm. per 100 cc. in terms of glucose. However glucid X is not properly described as a non-fermentable reducing substance, as it is sometimes destroyed by the briefest yeast fermentation[15] and is always completely fermented in seventy-two hours.[14] A preferable method of estimating glucid X is therefore by the difference between the reduction in copper and tungstate filtrates using the Folin-Wu reagents.

THE Y-REDUCTION

Comparing the non-glucose reduction by different methods Pickard, Pierce and associates found that the Benedict modification of the Lewis-Benedict reduction method (it is not fair to call it a method for blood sugar) gave regularly a much greater reduction in the blood than the Folin-Wu although the amount of the body or bodies which caused this reduction over that of the true glucose and glucid X determined by the Folin-Wu varied widely in different bloods. Calling the excess reduction the "Y-reduction," they found it ranged in the blood of twenty-five healthy, fasting students from 14 to 68 mgm. per 100 cc. an average of 40.4 mgm. per 100 cc. as glucose. In forty-one blood samples

* An equal quantity of a 5 per cent suspension of Fleischmann's yeast is added to whole blood, shaken, and allowed to stand. Although one minute is sufficient, five minutes is the usual period used. Washing the yeast has been shown to be unnecessary. Add distilled water to make 8 volumes, then precipitants and filter.

from patients in whom kidney damage or irritation was shown by the presence of albumin and casts in the urine the Y-reducing substances averaged 58 mgm. per 100 cc. in the four cases of severe nephritis included, they averaged 108 mgm.

The Y-reduction is not due to a fault in the picrate method of blood sugar analysis, since the Myers[10] blood sugar method was found (as is well known) to check accurately with the Folin-Wu, giving only the reduction due to glucose and to glucid X. Thus the Myers-Bailey method may be used with the Benedict modification of the Lewis-Benedict to estimate the Y-reduction. This control by the Myers test answers a question as to the cause of the excess reduction which is raised by the work of Duggan and Scott[3] who showed that with the Benedict modification the formation of picramic acid varied with both the glucose and the amount of proteins to be precipitated. The Y-reduction was also shown to have no relation to the creatinin bodies, nor to an increased sensitivity of the picric acid method to glucid X. In twenty-seven blood specimens from nephritics glucid X was normal, average 20.8 mgm. per 100 cc. The Y-reduction from these bloods showed no relation to glucid X (Y-reduction 18 to 136 mgm., average 55.5 mgm.).

The Y-reduction as a difference between two picric acid methods could be considered as more satisfactorily demonstrated as being due to the presence of a reducing substance in the blood than when left as a comparison with different precipitants and different oxidizing reagents. But it was desirable to show this reduction in other filtrates by a method as sensitive as the Benedict modification. This is possible by the Ionesco[11] method, also originally offered as a method for blood glucose. This method, like the Folin-Wu, shows no reduction even from large amounts of added creatinin, nor is it affected by yeast as used for blood sugar fermentation, either in filtrates from a yeast suspension, or from yeast added immediately after the precipitants to blood. It reduces with phenolphthalein and after acid hydrolysis the filtrate must be neutralized according to a control.*

* Reduction method of Ionesco-Matiu. In a 30 cc. Erlenmeyer flask (200 x 15 mm. tubes serve as well, and are more convenient in the water bath) put 5

In the tungstate filtrate the Ionesco method gives a reduction in excess of the Folin-Wu corresponding to that of the Benedict modification, so the substance causing the Y-reduction is present in the tungstate filtrate although it is not oxidized by the Folin-Wu reagents.[13] The reduction in the trichloroacetic acid filtrate for which the Ionesco method was originally recommended in some instances was higher than in the meta-phosphoric and tungstic acid filtrates. Trichloroacetic acid does not precipitate the polypeptids. Cristol[2] estimates these from the difference between the non-protein nitrogen in this filtrate and the non-protein nitrogen in the tungstate filtrate.

SIGNIFICANCE OF THE Y-REDUCTION

Pickard, Pierce and associates found the Y-reduction almost constantly increased in nephritis over the normal of 40 mgm. per 100 cc., estimating it by subtracting the apparent glucose of the Folin-Wu method from the reduction given by the Benedict modification of the Lewis-Benedict method. Pickard compared the Folin-Wu with the Ionesco methods in the same filtrate, and with the Benedict modification, the average Y-reduction from all bloods was 67.6 and 69.4 mgm. per 100 cc. respectively. That the Y-reduction showed a wide divergence in some bloods is not strange when the difference in the means of estimation is considered. This reduction is probably due to several substances which precipitation by picric or tungstic acids and the oxidation by picric acid, or by ferricyanide and permanganate, may affect even in opposite ways. The eight blood specimens from healthy

cc. of blood filtrate (equivalent to 0.5 cc. blood), 2 cc. normal NaOH, 1 cc. ferricyanide reagent, 10 cc. distilled water. Place in boiling water bath twelve minutes. Cool (the mixture should be light yellow). If colorless repeat using 2.5 cc. of filtrate. Add 5 cc. of a 20 per cent iron free H_2SO. The color changes to water green. From a micro-burette add N/60 $KMnO_4$ to form a pink tint. Each cubic centimeter of N/60 $KMnO_4$ is equivalent to 100 mgm. of glucose per cent, when using the filtrate from 0.5 cc. blood. Use a control blank, or with 5 cc. Folin-Wu weak standard glucose (without benzoic acid) and subtract the correction. The ferricyanide reagent is composed of potassium ferricyanide 23 grams, KOH 23 grams, distilled water to make 1000 cc. The ferricyanide reagent and N/10 $KMnO_4$ must be kept in the ice box; the permanganate keeps about three or four weeks.

individuals examined by Pickard had an average Y-reduction of 38 mgm. The variation[12] was wide varying from 5 to 75 mgm. per 100 cc. Eleven samples from nephritics averaged 66 mgm., the five severe cases included averaged 97 mgm. for the Y-reduction. (70–136 mgm. per 100 cc.) Two syphilitics gave 58 and 65 mgm., three female patients with anemia, and a hemoglobin average of 11 grams per 100 cc. gave a Y-reduction of 80 mgm. per 100 cc. Four of the patients with nephritis had an apparent blood sugar (Folin-Wu) over 120 mgm. (125–154 mgm. per 100 cc.), a higher blood sugar has long been noted as being common in

TABLE 1

ANALYSES OF BLOOD BY DIFFERENT METHODS IN VARIOUS CONDITIONS*

TYPE OF CASE	FOLIN-WU METHOD				IONESCO METHOD				Y-REDUCTION			
	Cells	Plasma	Whole blood	Serum	Cells	Plasma	Whole blood	Serum	Cells	Plasma	Whole blood	Serum
Nephritis	122	123			333	198			211	75		
Tetany		97	160			130	250			33	90	
After yeast fermentation		23†	25†			60	70					
Nephritis			100			100	170				70	
Nephritis			136		130		160	125			24	
Normal			113	100			134	127			21	27

* All results given in terms of milligrams per 100 cc.

† Glucid X.

nephritis. The substances giving rise to the Y-reduction are thus increased in nephritis and perhaps in anemic conditions, two conditions in which cellular metabolism is retarded. Table 1 gives a comparison of various reducing substances in the blood in different conditions as determined by different methods.

COMPARISON WITH GLUTATHION

Glutathion is the chief of the sulphydryl compounds that play an important part in tissue respiration forming an oxidation-reduction system in the cells. This auto-oxidizing power of glutathion depends on the presence of catalytic metals (iron,

copper) and an additional factor (Wurmser[20]), possibly existing in combination in the cells, a reason to which Fabre[5] attributes the difficulty of dissolving it in the tissues. Glutathion has been found in nearly all the tissues including the blood where it is confined to the cells and is said to be the chief non-glucose reducing body in whole blood. Everett[4] with a solution of glutathion equivalent to 100 mgm. per 100 cc. in the blood, got a reduction of 21 mg. as glucose with the Folin-Wu method. He found that zinc precipitated glutathion added to blood and also the substances forming part of the "hydrolysable sugar of the blood," and thought that the reducing substance which is conveniently named glucid X, was glutathion. There are a number of workers besides Best who have shown that glucid X is not glutathion. Fontès and Thivolle have shown that neither the SH nor the S-S groups affect their reagents, which oxidise glucose and glucid X and Somogyi[16] said that glutathion as it exists in the blood does not reduce alkaline copper solutions. Glutathion is present in the cells, glucid X is equally in cells and plasma. The substances causing the Y-reduction, is chiefly in the cells, do not react to the alkaline copper reagents, and might well comprise glutathion as the largest factor. Glucid X is completely fermented in three days, neither glutathion nor the substances causing the Y-reduction is fermented by yeast.

The average reduction of glutathion* in solutions of 50,100 and 200 mgm. per 100 cc. by the Folin-Wu method was 20 mgm. (as glucose), added to blood 21 per cent, the actual quantity being thus five times the amount recorded in terms of glucose. By the Ionesco method the reduction in pure solutions was 63 per cent, of the real amount as glucose; added to blood, only 49 per cent. Neither boiling nor acid hydrolysis affected either the Y-reduction of bloods nor the glutathion as regards either reducing power or the amount of nitrogen present. A solution of glutathion of 100 mgm. per 100 cc. gave a nitrogen content of 14 mgm., and when added to blood in the same amount the added non-protein nitrogen over that of the blood was on the average 13.8 mgm. This

* This was received through the courtesy of Drs. A. H. Sanford and E. C. Kendall of the Mayo Clinic.

agrees with the Kendall[9] formula for glutathion, and shows that glutathion added to blood is not precipitated by tungstic acid although there is a loss in reducing power with the Ionesco reagents. That the glutathion in the cells does not reduce the Folin-Wu copper reagent shows that the glutathion is in a different state from that chemically isolated.

Peters and Van Slyke[12] state that the undetermined nitrogen makes up about a third of the total of the blood and is largely confined to the cells. The amount of nitrogen from uric acid and

TABLE 2

METHODS FOR THE SEPARATION OF REDUCING BODIES IN THE BLOOD

TYPES OF FILTRATE	TRUE GLUCOSE (IMMEDIATELY FERMENTABLE)	GLUCID X (SLOWLY FERMENTABLE)	Y-REDUCTION (NOT FERMENTABLE)
Copper	**Folin-Wu**		
Tungstic acid	**Folin-Wu**		
	After the destruction of glucose by yeast	**Folin-Wu**	
		Ionesco	
	Ionesco		
Picric acid	**Myers-Bailey**		
Zinc	**Myers-Root**		
Picrate-picric acid	**Benedict modification of Lewis-Benedict**		

creatinin is so small as to be of little effect, the amino acid nitrogen is small and rarely changed in amount. Considerable changes in the non-protein nitrogen then, they say, are usually due to changes in the urea nitrogen, the undetermined nitrogen, or both.

On the assumption that the reduction of the glutathion added to blood estimated by the Ionesco method, is correct for that in the blood cells, the Y-reduction calculated as wholly glutathion should be double the figures in terms of glucose. Normal blood has a Y-reduction average of 40 mgm. per 100 cc. This gives 80 mgm. glutathion and 11 mgm. of nitrogen per 100 cc. Work

in progress shows that there are bloods in which this figure is too high. The Y-reduction in the plasma would also seem to be higher than compatible with a theory that was wholly caused by a substance freed from the blood cells. Other sulphydryl compounds, as ergothionine, may be included in the Y-reduction, as well as amino acids, the amino group of which is displaced by the $KMnO_4$ used to titrate the reduction of ferricyanide. Yeast contains glutathion, but the addition of even large amounts of yeast to sheep blood did not affect the Folin-Wu reduction, the "non-fermentable" glucid X figure remaining the same. After fermentation with yeast the Ionesco method shows the reduction of both glucid X and the Y-reduction. Boiling the yeast sets free reducing bodies which affect both methods in proportion to the volume of yeast, boiling a solution of glutathion did not change the reduction by either method, nor did boiling blood filtrates affect the reduction by the Ionesco method. Separation of the Y-reduction by Clausen treatment of the filtrate reported previously is erroneous.

SUMMARY

1. There is a reducing substance in the blood, chiefly confined to the cells, which gives a reduction by the Ionesco method in the tungstate filtrate in excess of the reduction of the Folin-Wu blood sugar method of an average of 40 mgm. per 100 cc. in normal blood. It may also be estimated as a comparison between other methods (table 2). It may conveniently be called the "Y-reduction."

2. As previously reported the Y-reduction is much greater in nephritis.

3. The Y-reduction is due to substances of both physiologic and pathologic interest, of which glutathion probably forms a large part.

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EDITORIAL

The Trouble with "Yaws"!

The term "yaws" is used here to designate the tropical condition which is supposed to be different from syphilis. The term "treponematosis" is used to include both syphilis, as it occurs throughout the world when recognized early and treated adequately, and yaws, which, as I view it, is syphilis operating under mediaeval conditions of personal hygiene.

Before 1500 A.D. treponematosis masqueraded under many names: leprosy, itch, mentagra, pudendagra, sycosis, psoriasis and a host of others. Epidemiologically, it is easy to see in the Great European Epidemic of Syphilis of the fifteenth century the condition which in many parts of the tropics is now called yaws. In spite of much contrary opinion, there have been many epidemics of this condition in many countries of the temperate zones during modern times.

Until about 1800 A.D. syphilis in most Anglo-Saxon countries was known as the greatpox, because it caused large and irregular scars upon its victims. This distinguished it from the smallpox, which scarred its victims with regularly placed small scars. During the fourteenth, fifteenth and sixteenth centuries practically everyone from the "Pope of Rome on his throne to the meanest scullion in Christendom" was scarred by one of these pocks.

In smallpox this scarring is accomplished not by the virus of variola per se but by vicarious bacteria, chiefly the tissue liquefying *Micrococcus aureus*. It is probable that *M. aureus* is also responsible for the major part of the mortality in variola, which is an acute disease, whereas syphilis is chronic. Otherwise the symbiotic action of the cocci is similar in the two diseases. When an initial lesion of syphilis appears, it may be heavily infected with extraneous organisms or comparatively free from them. In

either case *Treponema pallidum* invades the blood and tissues of the body while the cocci are held back by the host's defensive mechanism. When syphilides appear some are almost sure eventually to become superinfected with cocci and unless antiluetic treatment is instituted, these cocci will start pus formation, liquefaction of tissue and ultimate scarring in many lesions.

When the forces of liquefaction and of repair are fairly well balanced there is the histologic picture of a frambesioma, that is, of granulation tissue overlying a syphiloma. In syphilis this is noted in condylomatous, circinate and rupial types. In yaws the granulations give the strawberry appearance to the frambesioma (frambesia = strawberry) while vascular changes in the papillary layer, made necessary by the stimulus to repair, give the papillary projections upward and produce the corresponding interpapillary projections of epidermis downward. The characteristic polynuclear miliary abscesses are situated in these interpapillary pegs of epidermis. In yaws many of these eruptions are late frambesides in the congenital condition and hence will not have shown a "mother yaw."

If the juices of this lesion are sucked to the surface through this pathological filter bed a yellowish fluid reeking with microscopic life, one element of which is *Tr. pallidum*, is obtained. If a man be inoculated with this "polyvalent" antigen perhaps no lesion will appear for about three weeks or after a day or so the cocci will produce an ulcer or erosion which quickly heals and then later the chancre appears. Upon the skin this is usually not sclerotic but it may be so from the start or it may never be. If the flora of the inoculum is sufficiently virulent, the ulcerative and necrotic changes may start immediately and merge into the chancre when it appears.

After the second incubation period the secondaries come and the train of symptoms and sequelae known as syphilis. If, instead of man, the normal host of *Tr. pallidum*, some other animal is inoculated with the mixed antigen, the subsequent symptoms and course will depend entirely upon the species of animal and the method of inoculation. In any case, the symptoms are totally unlike syphilis in man.

It is as illogical as it is unscientific to inoculate this mixed antigen and then to conclude that the result is from just one element of that antigen. This, however, is what is being done in Manila. The highly contaminated material from cases of yaws inoculated into monkeys will produce symptoms in part due to the so called *Treponema pertenue* and in part to accompanying organisms. The results, however, are here interpreted as due to *Tr. pertenue* alone. Not only this but one is asked to accept for man the results obtained in this questionable manner and upon an animal not normally suffering from treponematosis. The science of medicine is in possession of adequate knowledge of the pathogeny of *Tr. pallidum* when operating in the tissues of man away from the surface of the body. In no case does it produce suppuration and liquefaction of tissue when operating in a pure state. Why, in the case of syphilis, does one discriminate in the interpretations of results and in the case of yaws remain blind to the scientific defects of experimentation.

In smallpox the total effect is due to several viruses—to the real virus of variola, which is unknown, and to cocci and other organisms. So in syphilis the scarring lesions are attributed to the combined effect of *Tr. pallidum* working underground and the contaminant liquefiers operating on the surface. It is a truism to state that if all cases of syphilis could be treated when the roseola appears, there would not be any other eruptions. Salvarsan is specific for Treponema but not for Micrococcus.

In yaws some do not make such distinctions. The skin lesions with monkey according to Schöbl are due to *Tr. pertenue* and not to the accompanying bacteriological "menagerie." Gangosa is definitely from yaws. Based on these experiments, one is asked to change his entire conception of yaws. Knowledge has been gained concerning the laws which govern the method of tissue invasion on the part of *Tr. pertenue* and its production of immunity. Even the long established terminology of yaws is changed to conform. But the trouble with this conception is that it not only violates the laws of epidemiology, but constitutes a personally-conducted, one-man, hand-picked conception of a disease which is unrecognizable from the standpoint of earlier investi-

gators. The skin lesions in the monkey have been played up at the expense of those visceral and osseous lesions which formed such a vital part of the clinical descriptions of those masters of the subject such as Winterbottom and Numa Rat, who painted pictures of human yaws. The seriousness of this constitutional disease as depicted by these investigators can not be "laughed off" by the "high interpretation" of a few score protocols upon monkeys and rabbits.

I have seen florid yaws around Manila Bay all the way from Parañaque to Cavite and have treated many cases of early and late yaws in the Naval Clinic for natives at Cañacao. I have also seen early and late yaws at many places in the West Indies, particularly in Haiti and LaGonave. I can assure readers of the American Journal of Clinical Pathology that the one differs in no wise from the other in the human being. The trouble with yaws as a distinct disease may be categorically stated thus; it simply does not explain the known facts in the epidemiology and pathology of treponematosis.

There is some interesting psychology shown by "yaws experts." Most of us have some certain means of differentiating yaws from syphilis, but we cannot explain this so that the "man from Missouri" can use it and yet we get irritated if anyone questions our infallibility. One investigator has a "dead shot" bone lesion about the knee joint that is given by syphilis and not by yaws or vice versa. Another will see in the fungating lesion of yaws "pushing up through the skin" an absolutely unique type of lesion peculiar to and distinctive of yaws. Such a writer will not believe that an exactly similar lesion occurs in untreated syphilis. Rupial, condylomatous and circinate lesions seen in neglected syphilis require no change in description to make them typical yaws eruptions in a filthy native of the tropics. Still a fourth authority deplores the fact that a certain distinguished author of textbooks on tropical medicine "finds himself practically convinced of the identity of yaws and syphilis." "If this be true," he continues, "then it is the first proved example of a disease which has a symptomatology in tropical regions not found elsewhere." This, in spite of the publication of scores of epidemics

and hundreds of sporadic cases from the syphilised populations of every parallel of latitude from the equator to the polar circles.

The most interesting bit of psychology in "yaws work," however, is that shown by medical editors. Almost universally they will turn down manuscripts which attempt to point out the glaring inconsistencies which stalk abroad everywhere in the yaws set-up. Usually the bogy they get behind is that of "controversial material." Many of these questions are not points of controversy but are good common sense and orthodox medicine. Granted, however, that they are controversial, what would Galen, the great controversialist think of such an alibi if he could return to life? What answer would be given to the statement of the fact that medicine has been one long controversy since its history began to be recorded? How many centuries behind its present exalted position among the learned professions medicine would be today had it not been for controversy, is not for me to say.

C. S. BUTLER.

NEWS AND NOTICES

The Eleventh Annual Convention of the American Society of Clinical Pathologists

The Committee on Local Arrangements and the Secretary take pleasure in announcing the following general program for the meeting to be held in New Orleans May 6 to 9, 1932. The Jung Hotel will be the headquarters for the Society and the program for the Scientific Session is:

Friday Morning, May 6

The autopsy and the pathologist.—Israel Davidsohn.

Evidence to support the contention that moles are of a neuroepithelial nature.—A. C. Broders and Eleanor M. Fletcher.

Histopathology of methyl alcohol poisoning.—Ernest Scott.

Less common tumors of the liver.—O. A. Brines.

Some essentials to satisfactory work in allergy.—J. H. Black.

Bronchial asthma and its treatment.—Bernard McCloskey.

Laboratory diagnosis of tuberculous meningitis.—A. G. Foord.

Friday Afternoon, May 6

The action of phenylhydrazine-hydrochloride and acetylphenylhydrazine with special reference to the use of these compounds in polycythemia.—M. Bodansky, W. L. Marr and Paul Brindley.

The agglutinin content of blood following typhoid and para-typhoid immunization.—A. G. Foord.

The blood picture in pneumonia with special reference to degenerative changes of the leukocytes.—N. Rosenthan and Charles Sutro.

Contribution to the clinical pathology of diabetes.—W. G. Exton.

Viability of tubercle bacilli. Effect of the chemical treatment used in concentration technic.—J. E. Pottenger.

Further observations on amebic infection of surgical wounds.—O. B. Hunter.

Experience with the hormone test for pregnancy.—F. E. Sondern and Jerome Silverman.

The results of two years' experiment with the Friedman test.—H. L. Reinhart.

Saturday Morning, May 7

The hematopoietic system and infection.—B. Markowitz.
Lymphomatous compression of the spinal cord.—F. H. Lamb.
Bone marrow pathology in leukopenic diseases.—R. R. Kracke.
A study of the frequency in occurrence of myeloid immaturity in pernicious anemia.—F. J. Heck.
The neutrophil in pernicious anemia.—F. J. Heck and C. H. Watkins.
Hemoglobin standards.—R. L. Haden.

Saturday Afternoon, May 7

Rose Bengal test in liver function technic and results.—W. Parker Stowe.
Liver function tests.—T. B. Magath.
A study of the so-called "O" and "H" agglutinins in typhoid and endemic typhus fevers.—H. Kemp.

Symposium

Should the precipitation test for syphilis be adopted to the exclusion of complement fixation procedures?—B. S. Levine.
The present status of the serological diagnosis of syphilis.—R. A. Kilduffe.
Discussion by: B. S. Levine and R. Gilbert.

In addition to a short business session on Friday morning the regular business session will be held Monday May 9 at 9 a.m.

A special feature of the program will be a complimentary Buffet Supper at the Jung Hotel Friday May 6, at 6:30 p.m. Following this supper there will be a Round Table Discussion of two important subjects: (1) The relation of the pathologist to the individual physician and to the hospital and its staff as a unit, (2) Hospital charges for clinical laboratory service. The Annual Banquet will be held on Saturday evening May 7 at the Patio Capital where Parisian chefs serve their apprenticeship. President Corpor will deliver his address on "Prospect and Retrospect" then and other speakers, yet to be announced, will address the society.

Of particular interest to the Society will be the unusual feature of a trip to the Leprosarium which is under the active supervision of Major O. E. Denney. Dr. F. M. Johns, Chairman of the Local Arrangements Committee has supplied the following interesting information concerning the Leprosarium:

While it is difficult to trace the exact origin of endemic leprosy in Louisiana, it is likely that large families of Spanish-French origin hide a sufficient number of chronic carriers to supply a considerable annual crop of acute leprous lesions that necessitate medical assistance. The strong religious nature of the Louisianians and the biblical references to the "unclean leper" undoubtedly stimulated the founding of the State institution which is operated by a Catholic nursing Sisterhood. The gradual spread of the disease and its development in other parts of the United States caused the Federal Government several years ago to take over the institution at Carville and convert it into a Federal Hospital. Dr. Denny who has been in charge for some time has made many notable contributions to the study of leprosy and has personally prepared the largest collection of color photographs of this disease. The Leprosarium is 90 miles above New Orleans on the Mississippi River, it was originally one of the aristocratic sugar plantations of ante-bellum fame and is picturesquely situated amid moss covered Louisiana water oaks.

The trip as planned will not only be of great medical interest but will carry the members of the Society through some of the most historic country in the United States. The party will leave in touring buses promptly at 9 a.m., Sunday morning and will travel across the Mississippi River and head west into the famed Louisiana bayou country where one may see not only the most modern sugar plantations of the State but some of the early iron crucibles which the negroes used more than a hundred years ago. Several typical Louisiana towns will be passed and many famous plantation homes will be seen, including that of Chief Justice White near Napoleonville. The party will be refreshed with luncheon as guests of Dr. Denney and will return by another route past the oil refineries and the new two mile wide spillway that protects New Orleans and will return to the Hotel about 7 p.m.

Tickets costing $5.00 each will be on sale at the registration desk and will be limited to members of the Society, their relatives and friends.

The Executive Committee will meet Saturday night at the Jung Hotel.

Although there have been many methods described for using brilliant cresyl blue in staining reticulocytes, the following sub-

mitted by Dr. John C. Simpson, Norristown, Pennsylvania will be found very useful:

(1) With a wax pencil draw a ring ½ inch in diameter near one end of a clean glass slide.

(2) Place a fair sized drop of 0.3 per cent alcohol solution of brilliant cresyl blue in the ring and allow it to dry in the air. The ring prevents the stain from spreading over the slide and becoming too thin. Slides prepared in this manner may be kept for many months and good results may be obtained even after as long as eight months.

(3) Touch the stain to a drop of fresh blood on the ear or finger and allow to stand until the blood turns black or dark blue. This takes place in from five to six seconds and before the blood clots.

(4) Smear the blood along the slide in the usual manner and stain with Wright's stain. No difficulty is experienced in drawing the blood across the wax pencil ring.

By this method the reticulations stand out very clearly as clumps of dotted blue lines in the erythrocytes.

Members of the A. S. C. P. should receive a great deal of satisfaction over the verdict rendered by the Court in the recent Baker trial at Davenport, Iowa. The jury found for the defendant, the American Medical Association. Much of the evidence presented upon which the decision was reached was based on the science of pathology and many members of the Society assisted the Association in presenting its side of the case.

Notice has been received of the death of Dr. George H. Fox of Binghamton, N. Y., and Dr. W. F. Thomson of Beaumont, Texas. Both were members of the Society for some time.

BOOK REVIEWS

Man and Microbes. By STANHOPE BAYNE-JONES. Pp. 128, 1932, Baltimore, The Williams & Wilkins Company, $1.00.

This is a popular exposition of the information which is common to all students of microbiology. Most of the material deals with bacteriology in non-technical language, and in an instructive and interesting manner. The object for which the book was prepared has seemingly been well served, namely: to give the lay reader as much reliable information as can be obtained in a book of this size, and to give it to him in language which is readily understood. Books of this type should also find their place on the high school bookshelf, since reading of them may help the student in a decision concerning his future work.

LUTHER THOMPSON

Neoplasms of Domesticated Animals. By WILLIAM H. FELDMAN. Pp. 410, 1932, Philadelphia and London, W. B. Saunders Co., $6.00.

In the editorial pages of the JOURNAL, attention has been called to the value and scope of comparative pathology. It was suggested that studies in comparative pathology would yield contrastive as well as comparative results.

In this book by Feldman, the first of its kind to be published in the English language, we have what may be termed a beginning of such a study for there is no recent volume published anywhere that gives a comprehensive treatment of the neoplasms of domesticated animals. For this reason the volume will be valuable to all students of veterinary medicine, to pathologists who are at all interested in the broader aspects of pathology and to all students of cancer.

The monograph contains not only a review of the scattered material published by others but the major portion of the text is

concerned with the author's experience which covers more than 600 cases. The tumors in these animals are clearly described.

The main feature of the book is the large number of excellent illustrations. Of these there are 193, all of which are original; a large share of these are photomicrographs of unusually high quality.

The scheme of classification followed is that of Mallory with some slight modification and additions.

Apparently the science of pathology as related to domestic animals has not advanced beyond the state of gross and microscopic study of specimens, for little seems to be known of the pathological changes produced on the animal body outside of the gross and histological changes in the tumor. The science of roentgenology, so extensively applied to the study and treatment of human neoplasms, has been but little used in the case of lower animals and but small reference is therefore made to its use.

The book contains a chapter on experimentally transmissible tumors and closes with a section dealing with the preservation of pathologic material.

It is to be hoped that this excellent monograph will stimulate others to study and publish their observations in this important and significant field.